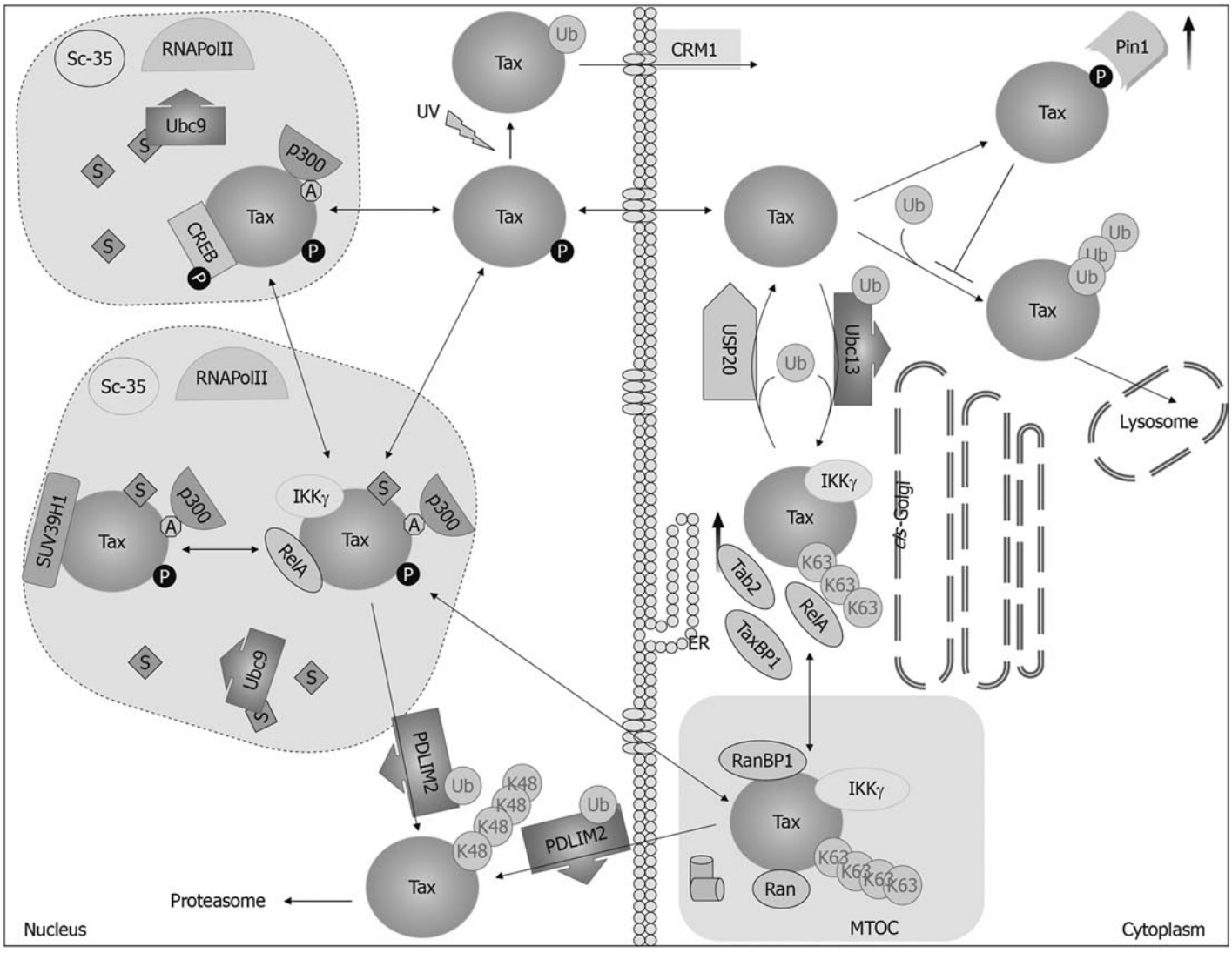
ISSN 2220-3249 (online)

World Journal of *Virology*

World J Virol 2012 August 12; 1(4): 108-130



Baishideng Publishing Group
www.wjgnet.com

World Journal of Virology

A peer-reviewed, online, open-access journal of virology

Editorial Board

2011-2015

The *World Journal of Virology* Editorial Board consists of 137 members, representing a team of worldwide experts in virology. They are from 41 countries, including Argentina (2), Australia (4), Austria (1), Barbados (1), Belgium (1), Brazil (2), Bulgaria (1), Canada (4), Chile (1), China (24), Croatia (1), Egypt (1), Finland (2), France (5), Germany (2), Greece (1), Hungary (1), India (3), Iran (1), Ireland (1), Israel (2), Italy (13), Japan (6), Kazakhstan (1), Mexico (1), Netherlands (3), Pakistan (1), Palestine (1), Poland (1), Portugal (1), Romania (1), Saudi Arabia (1), Singapore (1), Slovakia (1), South Africa (1), Spain (8), Sweden (1), Thailand (4), Turkey (1), United States (28), and Uruguay (1).

WJV

World Journal of Virology

Contents

Bimonthly Volume 1 Number 4 August 12, 2012

World Journal of Virology
Volume 1 Number 4 August 12, 2012

Contents

NAME OF JOURNAL
World Journal of Virology

ISSN
ISSN 2220-3249 (online)

LAUNCH DATE
February 12, 2012

FREQUENCY
Bimonthly

EDITING
Editorial Board of *World Journal of Virology*
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: wjv@wjgnet.com
http://www.wjgnet.com

EDITOR-IN-CHIEF
Ling Lu, MD, PhD, Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, 3901 Rainbow Blvd, WHE 3020, KS 66160, United States

EDITORIAL OFFICE
Jian-Xia Cheng, Director
Jin-Lei Wang, Vice Director
World Journal of Virology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-8538-1893
E-mail: wjv@wjgnet.com
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Co., Limited
Room 1701, 17/F, Henan Building,
No.90 Jaffe Road, Wanchai, Hong Kong, China
Fax: +852-31158812
Telephone: +852-58042046
E-mail: bpg@baishideng.com
http://www.wjgnet.com

PUBLICATION DATE
August 12, 2012

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/2220-3249/g_info_20100722180909.htm

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Virology

Online Submissions: http://www.wjgnet.com/esps/
wjv@wjgnet.com
doi:10.5501/wjv.v1.i4.108

World J Virol 2012 August 12; 1(4): 108-114
ISSN 2220-3249 (online)

EDITORIAL

History of polio vaccination

Anda Baicus

Anda Baicus, National Institute of Research and Development for Microbiology and Immunology Cantacuzino, University of Medicine and Pharmacy "Carol Davila", 050096 Bucharest, Romania
Author contributions: Baicus A solely contributed to this paper.
Correspondence to: Anda Baicus, MD, PhD, Lecturer in Microbiology, Head of the National Polio Laboratory, National Institute of Research and Development for Microbiology and Immunology Cantacuzino, University of Medicine and Pharmacy "Carol Davila", 050096 Bucharest, Romania. abaicus@cantacuzino.ro
Telephone: +40-740-213102 Fax: +40-215-287305
Received: October 11, 2011 Revised: June 12, 2012
Accepted: July 13, 2012
Published online: August 12, 2012

Abstract

Poliomyelitis is an acute paralytic disease caused by three poliovirus (PV) serotypes. Less than 1% of PV infections result in acute flaccid paralysis. The disease was controlled using the formalin-inactivated Salk polio vaccine (IPV) and the Sabin oral polio vaccine (OPV). Global poliomyelitis eradication was proposed in 1988 by the World Health Organization to its member states. The strategic plan established the activities required for polio eradication, certification for regions, OPV cessation phase and post-OPV phase. OPV is the vaccine of choice for the poliomyelitis eradication program because it induces both a systemic and mucosal immune response. The major risks of OPV vaccination are the appearance of Vaccine-Associated Paralytic Poliomyelitis cases (VAPP) and the emergence of Vaccine Derived Polioviruses strains. The supplementary immunization with monovalent strains of OPV type 1 or type 3 or with a new bivalent oral polio vaccine bOPV (containing type 1 and type 3 PV) has been introduced in those regions where the virus has been difficult to control. Most countries have switched the schedule of vaccination by using IPV instead of OPV because it poses no risk of vaccine-related disease. Until 2008, poliomyelitis was controlled in Romania, an Eastern European country, predominantly using OPV. The alternative vaccination schedule (IPV/OPV) was implemented starting in September 2008, while beginning in 2009, the vaccination was IPV only. The risk of VAPP will disappear worldwide with the cessation of use of OPV. The immunization for polio must be maintained for at least 5 to 10 years using IPV.

Key words: Poliomyelitis; Formalin-inactivated polio vaccine; Oral polio vaccine

Peer reviewer: Ian M Mackay, PhD, Qpid Laboratory, Sir Albert Sakzewski Virus Research Centre, Royal Children's Hospital, Herston Road, Herston Q4029, Australia

Baicus A. History of polio vaccination. *World J Virol* 2012; 1(4): 108-114 Available from: URL: http://www.wjgnet.com/2220-3249/full/v1/i4/108.htm DOI: http://dx.doi.org/10.5501/wjv.v1.i4.108

INTRODUCTION

Poliovirus (PV), an enterovirus belonging to the Picornaviridae family is the etiological agent of poliomyelitis, an acute paralytic disease. This disease results from lower motor neuron damage and is characterized by asymmetric persisting weakness (flaccid paralysis). The transmission of this virus during ancient times was suggested after the studies on Egyptian mummies, which showed a shortening of a lower limb in a child. In 1789, in the second edition of *A Treatise on Diseases of Children*, Michael Underwood described the disease as "debility of the lower extremities in children"[1]. He did not record any reference to outbreaks of this disease.

Badham[2] described an acute paralysis suggestive of poliomyelitis in four children in 1835. In 1840, Heine[3] published a monograph where poliomyelitis was recognized and defined as infantile spinal paralysis. Duchenne in 1855, then Charcot *et al*[4] in 1870, located the atrophy in the anterior horns of the spinal grey matter. This find-

ing gave rise to the pathological term "poliomyelitis" from the Greek *polios* for "gray" and *myelos* for "spinal cord". In 1875, Erb introduced the term "acute anterior poliomyelitis". Medin first reported the epidemic form of this disease in 1890, after an epidemic of 44 cases in Stockholm in the summer of 1887. He recognized a systemic phase of the disease which often failed to progress to neurological paresis and developed the classification of that. Wickman introduced, in 1907, the eponym "Heine-Medin disease" to honor Medin's contributions. Putnam *et al*[5] recorded the earliest descriptions of epidemic poliomyelitis using basic epidemiological methods in 1893, by Caverly[6] in 1894 in the United States and by Wickman in 1905 in Sweden. By the epidemiological studies conducted between 1910 and 1912 during epidemics in the United States, Frost found a widespread exposure to poliomyelitis but a low incidence of clinical disease to those susceptible to infection. During the epidemic in the north eastern United States in 1916, the role of asymptomatic persons in the spreading of infection was recorded by the Public Health Service. This epidemic caused widespread panic; over 27 000 persons were reported to have been paralyzed, with 6000 deaths.

The polio outbreaks gradually became more severe, more frequent and widespread throughout Europe and the United States at the beginning of the 20th century. The epidemiology of PV was gradually understood. The model of polio spread was irregular and many patients had no direct contact with a known source. In 1905, Wickman first recognized that poliomyelitis was an infectious disease. Landsteiner and Popper demonstrated in 1909 that the etiological agent of poliomyelitis was a filterable virus. They transmitted the disease to a Cynocephalus monkey by intraperitoneal injection of neural tissue from a human fatal case. In 1910, Flexner supposed that the PV was strictly neurotropic. He thought that PV entered the human body via respiration[7], a hypothesis that was later disproved. Howe and Bodian considered the possibility of the oral alimentary route of polio infection during the 1930[8]. In the late 1930s, Armstrong produced experimental poliomyelitis in mice. It was an advantage for the study of PV and for the development of a neutralization test in order to measure the antibodies. The assumption that there is more than one type of PV was launched by Burnet and Macnamara in 1931 and confirmed by Paul and Trask by observation in monkey experiments. The three distinct types were identified by a prototype strain, Brunhilde (type Ⅰ), Lansing (type Ⅱ) and Leon (type Ⅲ)[9]. The concept of poliomyelitis as an enteric infection had begun in 1932, when Paul and Trask found the virus in feces and recovered virus over a period of weeks from patients and healthy contacts. In 1936, Sabin and Olitsky reported that PV could be successfully grown *in vitro* in human embryonic neuronal tissue fragments cultivated in glass vessels. By sewage water testing in New York during periods when paralytic polio was prevalent, a ratio of 100 subclinical infections for every paralytic case was estimated[10]. In the 1950s, it was established that PV could be isolated from flies collected during epidemics. By the studies in the laboratory with flies emerging from maggots, no evidence of virus multiplication was recorded[11]. In 1949, Enders, Weller and Robbins successfully cultured the Lansing strain in nonneuronal tissue culture, leading to the capacity to produce the virus safely and in sufficient quantity, thus opening the way for production of viral vaccines. The serological studies about serum antibodies against PVs in underdeveloped countries showed that once exposed and infected, even asymptomatic persons were immunized for life. In developed countries, children living in crowded areas acquired infection at an earlier age than children from higher socioeconomic levels. By 1952, serological studies established that the antibodies against PV were present in the patient shortly after the onset of the disease and increased during convalescence. In 1952, a field trial conducted by Hammon showed that passive immunization by γ globulin administration assured protection against disease for only about 2 to 5 wk. Summer transmission of infection was associated with increased quantities of PV in sewage water. Two phases of disease, the gastrointestinal infection followed by viremia, and CNS invasion were confirmed by laboratory studies.

DEVELOPMENT OF PV VACCINES

In 1935, Brodie tried an inactivated vaccine with 10% formalin suspension of PV taken from infected monkey spinal cord; he tried it first on 20 monkeys, then on 3000 Californian children. The results were poor and additional human studies were never performed. In the same year, Kollmer tried a live attenuated virus consisting of a 4% suspension of PV from infected monkey spinal cord, treated with sodium ricinoleate. He used it on monkeys and then on several thousand children. The acute paralysis occurred in about 1/1000 vaccines shortly after administration and some cases were fatal.

The discovery that the various antigenic strains of PVs could be grouped into three distinct viral types and the propagation of the PV *in vitro* led to the development of the vaccines against poliomyelitis: the formalin-inactivated vaccine (IPV) by Jonas Salk (1953) and the live-attenuated vaccines (OPV) by Albert Sabin (1956)[12].

IPV

The first inactivated polio vaccine (IPV) was produced by Salk using virus grown on monkey kidney cells and inactivated with formalin. In 1954, the inactivated vaccine was tested in a placebo-controlled trial, which enrolled 1.6 million children in Canada, Finland and the United States[13]. In April 1955, Salk's vaccine was adopted throughout the United States. The incidence of paralytic poliomyelitis in the United States decreased from 13.9 cases per 100 000 in 1954 to 0.8 cases per 100 000 in 1961[14]. Some disadvantages of the Salk vaccine in that time were the decrease of the titres of the circulating an-

tibody within a few years of vaccination, the further circulation of wild PV and its implications in outbreaks, and the large number of monkeys (about 1500) needed to be sacrificed to produce every 1 million inactivated doses. The strains of virus used in the vaccine were Mahoney (type l), MEF-I (type 2) and Saukett (type 3). Shortly after the licensing of Salk vaccine, the failure of inactivation of vaccine virus at Cutter Laboratories, Berkeley, was followed by 260 cases of poliomyelitis with PV type 1 and 10 deaths. The supposition was that the virus was resistant to inactivation by formaldehyde because it contained more foreign proteins than optimal or that the virus may have clumped. A second filtration step was introduced in the production process in order to remove aggregates that may have developed during treatment and safety tests were improved. The use of the highly virulent Mahoney strain in vaccine production has been controversial and after the Cutter incident, even more so. In Sweden, the Brunenders strain for type 1 was preferred. In 1980, concentration and purification of polio antigens were introduced into the manufacture of IPV and the immunogenicity of the vaccine was increased. The original IPV contained 20, 2 and 4 D antigen units of PV types 1, 2 and 3. Van Wezel introduced a technology to produce enhanced potency IPV. He decided to concentrate and purify the virus before treatment with formalin. Since this procedure has been introduced, no failure in the inactivation process has been recorded. By the introduction of a new culture technique using cells on microcarrier beads in suspensions cultured in large stainless steel tanks, a more potent IPV containing 40, 8 and 32 D antigen units of types 1, 2 and 3 was produced[15,16]. Trials with this enhanced IPV (eIPV) showed greater than 90% seropositivity against all 3 PV types after one dose and 100% seropositivity after two doses[17]. An enhanced-potency IPV was licensed in the United States in 1987, with a good response to types 1 and 3 for either a 4 or 8 wk interval between doses and after 8 wk to type 2. The recommended schedule of vaccination in different countries contains 4 IPV doses in the primary series received at 2, 4, 6 and 15-18 mo of age[18] and a booster dose at age 4-6 years. For an optimum booster response, a minimum interval of at least 6 mo is needed between dose 4 and 5. In IPV vaccines, pharyngeal infection by PV is inhibited and intestinal excretion is reduced.

OPV

The development of the attenuated PV vaccine starts with passages of PV strains in rats and mice followed by passages in the cell culture. The reduction of the virulence of the PV strains was recorded in 1946 by Theiler, who passaged the Lansing strain in rats and mice more than 50 times and by Enders, Weller and Robins, who passaged the same strain in cell culture.

Candidate strains of attenuated PV suitable for immunizing humans were developed independently in the United States by Koprowski (Wistar Institute, Philadelphia), Cox (Lederle Laboratories) and Sabin (the Children's Hospital Research Foundation).

In 1950, Koprowski began experiments with a rodent-adapted type 2 PV that had been fed to a small group in California. He, Cox and their associates had fed millions throughout the world with the three types of viruses[19]. The isolation of PV with the properties of the Cox strain from the brain tissue of the dead father of a vaccinated child was followed by the withdrawal of this strain[20].

In 1960, Sabin described, in an article published in *JAMA*, *Live, orally given poliovirus vaccine*[21], the results obtained with his newly developed trivalent oral vaccine to 26 033 children from a city of 100 000 people in South America. Because the strains developed by Sabin provided good antibody levels and were less neurotropic for monkeys, they were selected and licensed between 1961 and 1963 in the United States for widespread application. The first nationwide polio vaccination campaign was in Cuba, in 1962[22]. During a meeting in 1956 between Sabin and Chumakov, Sabin provided his experimental results and his strains of polio vaccine to Chumakov, who began to produce it for use in his country. A few million children from Estonia and Lithuania (part of the Soviet Union at that time) received this vaccine by 1959 and it was a success story that contributed to the recommendation for license of the three monovalent strains developed by Sabin[23].

Sabin's OPV consists of three live attenuated Sabin poliovirus strains, obtained by sequential *in vitro* and *in vivo* passages of the wild strains. The virulent strains P1/Mahoney/41, P2/P712/56 and P3/Leon/37 served as a source for the attenuated Sabin strains: P1/Lsc,2ab, P2/P712,Ch,2ab and P3/Leon,12a$_1$b.

At the beginning, the trivalent OPV contained the three PV types in equal proportions but lower seroconversion rates to types 1 and 3 were recorded. By using a balanced formulation of trivalent OPV which contained 10^6, 10^5 and $10^{5.5}$ TCID50 (50% tissue culture infective dose) of Sabin types 1, 2 and 3, the neutralizing antibodies against all three PV types were detected in almost all persons. Increasing the amount of type 3 virus in the trivalent vaccine improved the immunogenicity[24] and the Expanded Program on Immunization Global Advisory Group recommended a formulation of trivalent OPV which contained 10^6, 10^5, $10^{5,8}$ TCID50 of Sabin types 1, 2 and 3 per dose[25]. The OPV vaccine was easier to administrate and had a herd effect, inducing long-lasting protective systemic, humoral and cellular immunity as well as local mucosal resistance to PV infection. In 1972, Sabin donated his vaccine strains of PV to the World Health Organization (WHO), increasing the availability of this vaccine to developing countries. From 1977 to 1995, the percentage of all children in the world who received the required three doses of OPV in the first years of life increased from 5% to 80%.

The major risks of OPV vaccination are the appearance of Vaccine-Associated Paralytic Poliomyelitis cases (VAPP) and the emergence of Vaccine Derived Polio-

viruses strains (VDPV), the OPV strains having more than 1% nucleotide divergence from the original vaccine strains in the VP1 coding region of the genome. The appearance of VAPP cases is due to the reversion to neurovirulence of the vaccine strains. During replication in intestine, the OPV strains can undergo genetic variation by point mutations at an average frequency of 10^{-4} due to RNA polymerase or through natural recombination. The incidence of VAPP for immunocompetent children receiving their first dose of OPV was estimated at one case per 750 000 doses and one case per 6.9 million subsequent doses[26]. Type 3 was the most common isolate associated with paralysis in vaccine recipients and type 2 was associated with paralysis mostly among contacts of cases. The VDPV strains could be circulant (cVDPV, which can spread in populations with low level of vaccine coverage), could emerge after replication in immunodeficient persons exposed to OPV (iVDPV), or could be ambiguous VDPV (aVDPV, when they are isolated from immunocompetent persons or the environmental source has not been identified). One dose of OPV produces immunity against all three PV serotypes in approximately 50% of recipients; three doses produce immunity in more than 95% of recipients.

MIXED IPV AND OPV

To eliminate the risk of VAPP among OPV vaccine recipients, the sequential schedule IPV/OPV was used in the world. However, the preimmunization of infants with two doses of IPV at 2 and 4 mo followed by two doses of OPV delivered at 18 mo and at 4 to 6 years did not eliminate the risk of VAPP among contacts of vaccines.

POLIO VACCINATION IN ROMANIA

In Romania, an Eastern European country, between 1927 and 1960 the evolution of poliomyelitis was sporadic and epidemic. The incidence of paralytic poliomyelitis in Romania decreased from 10 cases per 100 000 in 1949 to 0.1 cases per 100 000 by the mid 1980s by using inactivated PV vaccine in 1957 and oral PV vaccine in 1961. Polio vaccination started in 1957, with Lepine vaccine manufactured by the Pasteur Institute of Paris, but did not include the whole infant population (or even the entire contingent of children born in that year) and was followed in 1959 by IPV produced in USSR, with which all babies were vaccinated (two doses at a 2 mo interval). The success of the use of OPV, which determined the elimination of polio epidemics in many countries, led to the decision to use this new type of polio vaccine in Romania. First, the vaccine was bought from USSR and the entire Romanian population under the age of 30 years received this vaccine between 1961 and 1962. Cantacuzino Institute of Bucharest began the production of OPV in 1962, first using the vaccine seed virus received from the Poliomyelitis Research Institute in Moscow and later (since 1967), the vaccine seed virus was received directly from Sabin. Between 1961 and 1963, infants received a first dose of IPV and then OPV. Beginning in 1964, the trivalent oral vaccine stabilized with magnesium chloride prepared by the Cantacuzino Institute was used for widespread immunization in Romania. The administration of oral polio vaccine took place in annual national campaigns from 1961-1978. A few years after OPV was widely used, the incidence of paralytic poliomyelitis declined dramatically but VAPP cases began to appear. In 1974, the WHO approved the Cantacuzino Institute as an OPV production facility. Since 1970, Romania has participated for over 15 years with 11 other states on a WHO collaborative study on the risk of VAPP cases and the risk was the highest in this country. The health authorities from Romania decided in 1978 to stop the use of existing vaccine stocks (prepared with WHO seed viruses received in 1974) and to use a new WHO-B virus seed (Behring). This seed virus was to be available in summer 1978 but due to problems with preparation and especially control of seed, WHO-B viruses were distributed by the WHO only beginning at the end of 1979. Due to this problem, between July 1978 and March 1980, vaccination was only with monovalent OPV type 1 with a single dose administered to children aged 6 wk. During this period, no case of paralytic poliomyelitis was recorded in Romania. The vaccination was resumed with existing vaccine stocks in April-June. The effects of this 2-year disruption were epidemics which occurred during 1980-1982, caused by wild PV type 1 (161 cases) and type 2 (15 cases). The epidemic disappeared in the third quarter of 1982. The spreading of the wild PVs was stopped by an immunization campaign with trivalent OPV (TOPV). The poliomyelitis epidemic in Romania from 1980-1982 allowed an important conclusion: the interruption of TOPV vaccination for 2 years was sufficient to build a contingent of children highly susceptible to infection with PV, allowing re-implantation of wild PVs and their active movements, followed by the emergence of a polio epidemic. In 1983, vaccination campaigns were introduced in spring and fall. The vaccination schedule was designed so that each child should receive 4 doses TOPV in the first 10 years of life; first dose between 2 and 7 mo, second dose between 4 and 9 mo, third dose between 10-15 mo and a booster at 9 years of life. The vaccination coverage with three doses of TOPV at age 2 years was more than 90%. From 1983 to May 1990, no cases of paralytic poliomyelitis caused by wild PV were recorded. The latest outbreak of wild type 1 PV occurred between November 1990 and April 1992. The outbreak involved children from a gypsy community who were unvaccinated or inadequately vaccinated. Four of 13 wild PV cases were infected with HIV[27]. All cases of paralytic polio that occurred in Romania from 1984-1992 occurred in children younger than 5 years, which demonstrated that immunity to the three serotypes of PV was almost 100% for children older than 5 and adults.

The oral PV vaccine administered in Romania until September 1990 was produced by the Cantacuzino Insti-

tute. Because of a high rate of vaccine associated paralytic poliomyelitis reported from 1970-1984, beginning in November 1990, an oral vaccine approved by the WHO was imported and replaced the Romanian produced vaccine[28]. However, a case-control study demonstrated that the cause of elevated risk of vaccine associated paralytic poliomyelitis in Romania was not the PV vaccine manufacturer but the administration of multiple intramuscular injections of antibiotics within 30 d of receipt of OPV, which increased the risk of paralysis by a factor of 2 to 10 fold[29]. A decrease of the risk of VAPP was obtained by a reduction of parenteral treatment in recipients of OPV and by the change of the administration schedule of oral polio vaccine from 2 campaigns of two rounds, to throughout the year vaccination since April 1995. From 1992-1994, a project of the Ministry of Health supported by the Marcel Merieux Foundation demonstrated the feasibility, safety and high immunogenicity of sequential use of enhanced-potency IPV followed by OPV in 1 of the 41 counties in Romania[30]. Until 2008, poliomyelitis was controlled in Romania by predominantly using OPV administered at 2, 4, 6, 12 mo and a booster at 9 years of life; the alternative vaccination schedule (IPV/OPV) was implemented starting in September 2008 and at the beginning of 2009, vaccination with IPV only was decided[31]. The reported vaccination coverage with 4 doses of TOPV in the first 14 mo of life has been more than 90% since 1980. The risk of VAPP decreased from less than 2 VAPP cases per year in 1995-2006, to 0 VAPP cases since 2007[32]. In 2002, 1 mo after Certification of European region as polio free, a type 1 PV strain, aVDPV recombinant Sabin1/Sabin2/Sabin1 was isolated from a VAPP case not vaccinated against poliomyelitis and from 8 healthy contacts considered to be at risk[33]. In 2008, we studied the circulation and the biodiversity of enteroviruses in a group of children from a minority population with low anti-polio vaccination coverage from the same area where in 2002 a VDPV strain was isolated. Evidence of inter-human circulation of Sabin strains was found[34] but no VDPV strain was isolated. The surveillance of at risk populations from at risk areas and maintenance of complete vaccine coverage in the population are important objectives in the framework of global polio eradication.

CONCLUSION

With the development and use of vaccines, the complete eradication of poliomyelitis became an objective. In 1988, the WHO proposed the worldwide poliomyelitis eradication to its member states. The Global Polio Eradication Initiative (GPEI) Strategic Plan established the activities required for polio eradication, certification for regions, OPV cessation phase and post-OPV phase. At the beginning, this plan was based on maintaining of a high vaccination coverage (> 80%) among children, the application of supplementary vaccine doses during national vaccination days (NVD), mopping up vaccination and implementing effective PV infection surveillance systems and containment activities. The presence of susceptible subgroups with gaps in immunization favors the introduction of wild PV strains in a vaccinated population. The OPV strains have become the main instrument for the wild-type PV eradication program because it induces both a systemic and mucosal immune response. Most countries have switched the schedule of vaccination against polio by using IPV instead of OPV. The advantage of using IPV is that it poses no risk of vaccine-related disease. The disadvantages for the global introduction of IPV are its cost, the intramuscular administration, its inability to produce optimal intestinal immunity and the biocontainment required for its production. In 2011, 23 years after the decision of the WHO to globally eradicate poliomyelitis, the wild PV (type 1 and 3) is still endemic in only four countries: Afghanistan, India, Nigeria and Pakistan. The type 2 wild PV strain has been eradicated globally since 1999, while a type 2 circulating vaccine-derived PV (cVDPV) has persisted in northern Nigeria since 2006[35]. A plan for the cessation of routine OPV immunization against type 2 PVs must be devised[36]. In those regions where the virus has been difficult to control, supplementary immunization with monovalent strains of OPV type 1[37] or type 3[38], or with new bivalent oral polio vaccine bOPV (containing type 1 and type 3 PV) has been introduced[39]. In 2008, the GPEI plan was updated in order to overcome barriers to interruption of wild PV transmission. The objectives for 2010-2012 are: interrupting wild PV transmission in Asia and Africa; enhancing PV surveillance and outbreak response; and strengthening immunization systems. In 2010, an outbreak of wild PV type 1 cases genetically related to wild PV circulating in 2009 in India was recorded in Tajikistan, part of the WHO European Region certified polio-free in 2002[40]. This episode demonstrated that if a region is polio free, the risk of wild PV importation from endemic regions remains present until polio is globally eradicated. Because most VDPV strains implicated in poliomyelitis outbreaks worldwide are recombinants between OPV strains and non polio enterovirus strains of Human enterovirus species C[41], increasing the surveillance of co-circulation and evolution of polio and non-polio enteroviruses must be achieved. The risk of VAPP will disappear with the cessation of use of OPV. Some research programs are initiated by WHO for obtaining an affordable IPV by reduction of the necessary antigen dose by intradermal administration[42,43], by using adjuvants[44] and by introduction of Sabin strains as seed[45,46]. In the first 5 to 10 years after global cessation of OPV administration, the maintenance of immunity to polio by IPV use must be assured[47].

REFERENCES

1 **Underwood M**. Debility of the lower extremities. In: Treatise on the diseases of children. London: J Mathews, 1789: 53-57
2 **Badham J**. Paralysis in childhood: four remarkable cases of suddenly induced paralysis in the extremities without any

apparent cerebral or cerebrospinal lesion. *London Med Gaz* 1835; **17**: 215-218
3 **Heine J**. Beobachtungen uber lahmungustande der untern extremitaten und deren behandlung. Stuttgart: FH Kohler, 1840
4 **Charcot JM**, Joffroy A. Une observation de paralysie infantile s'accompagnant d'une alteration des cornes anterieures de la substance grise de la moelle. *CR Soc Biol* (Paris) 1870; **1**: 312-315
5 **Putnam JJ**, Taylor EW. Is acute poliomyelitis unusually prevalent this season? *Boston Med Surg J* 1893; **129**: 509-510
6 **Caverly CS**. Preliminary Report of an Epidemic of Paralytic Disease Occurring in Vermont, in the Summer of 1894. *Yale Med J* 1894; **1**: 1-5
7 **Flexner S**, Lewis PA. Experimental poliomyelitis in monkeys: active immunization and passive serum protection. *JAMA* 1910; **54**: 1780-1782
8 **Melnick JL**. Current status of poliovirus infections. *Clin Microbiol Rev* 1996; **9**: 293-300
9 **Paul JR**. A history of poliomyelitis. New Haven, CT: Yale University Press, 1971
10 **Melnick JL**. Poliomyelitis virus in urban sewage in epidemic and in nonepidemic times. *Am J Hyg* 1947; **45**: 240-253
11 **Melnick JL**. My role in the discovery and classification of the enteroviruses. *Annu Rev Microbiol* 1996; **50**: 1-24
12 **Sabin AB**, Boulger LR. History of Sabin attenuated poliovirus oral live vaccine strains. *J Biol Stand* 1973; **1**: 115-118
13 **Monto AS**. Francis field trial of inactivated poliomyelitis vaccine: background and lessons for today. *Epidemiol Rev* 1999; **21**: 7-23
14 **Strebel PM**, Sutter RW, Cochi SL, Biellik RJ, Brink EW, Kew OM, Pallansch MA, Orenstein WA, Hinman AR. Epidemiology of poliomyelitis in the United States one decade after the last reported case of indigenous wild virus-associated disease. *Clin Infect Dis* 1992; **14**: 568-579
15 **van Wezel AL**, van Steenis G, van der Marel P, Osterhaus AD. Inactivated poliovirus vaccine: current production methods and new developments. *Rev Infect Dis* 1984; **6** Suppl 2: S335-S340
16 **Montagnon BJ**, Fanget B, Vincent-Falquet JC. Industrial-scale production of inactivated poliovirus vaccine prepared by culture of Vero cells on microcarrier. *Rev Infect Dis* 1984; **6** Suppl 2: S341-S344
17 **Bernier RH**. Improved inactivated poliovirus vaccine: an update. *Pediatr Infect Dis* 1986; **5**: 289-292
18 **von Magnus H**, Petersen I. Vaccination with inactivated poliovirus vaccine and oral poliovirus vaccine in Denmark. *Rev Infect Dis* 1984; **6** Suppl 2: S471-S474
19 **Centers for Disease Control and Prevention (CDC)**. Progress toward global eradication of poliomyelitis, 1988-1993. *MMWR Morb Mortal Wkly Rep* 1994; **43**: 499-503
20 **Cook JS**, Wolsing DH, Lameh J, Olson CA, Correa PE, Sadee W, Blumenthal EM, Rosenbaum JS. Characterization of the RDC1 gene which encodes the canine homolog of a proposed human VIP receptor. Expression does not correlate with an increase in VIP binding sites. *FEBS Lett* 1992; **300**: 149-152
21 **Sabin AB**, Ramos-Alvarez M, Alvarez-Amezquita J, Pelon W, Michaels RH, Spigland I, Koch MA, Barnes JM, Rhim JS. Landmark article Aug 6, 1960: Live, orally given poliovirus vaccine. Effects of rapid mass immunization on population under conditions of massive enteric infection with other viruses. By Albert B. Sabin, Manuel Ramos-Alvarez, José Alvarez-Amezquita, William Pelon, Richard H. Michaels, Ilya Spigland, Meinrad A. Koch, Joan M. Barnes, and Johng S. Rhim. *JAMA* 1984; **251**: 2988-2993
22 **Hinman AR**. Landmark perspective: Mass vaccination against polio. *JAMA* 1984; **251**: 2994-2996
23 **Agol VI**, Drozdov SG. Russian contribution to OPV. *Biologicals* 1993; **21**: 321-325
24 **Patriarca PA**, Laender F, Palmeira G, Oliveira MJ, Lima Filho J, Dantes MC, Cordeiro MT, Risi JB, Orenstein WA. Randomised trial of alternative formulations of oral poliovaccine in Brazil. *Lancet* 1988; **1**: 429-433
25 Expanded programme on immunization. Global Advisory Group--Part II. *Wkly Epidemiol Rec* 1991; **66**: 9-12
26 **Prevots DR**, Sutter RW, Strebel PM, Weibel RE, Cochi SL. Completeness of reporting for paralytic poliomyelitis, United States, 1980 through 1991. Implications for estimating the risk of vaccine-associated disease. *Arch Pediatr Adolesc Med* 1994; **148**: 479-485
27 **Strebel PM**, Aubert-Combiescu A, Ion-Nedelcu N, Biberi-Moroeanu S, Combiescu M, Sutter RW, Kew OM, Pallansch MA, Patriarca PA, Cochi SL. Paralytic poliomyelitis in Romania, 1984-1992. Evidence for a high risk of vaccine-associated disease and reintroduction of wild-virus infection. *Am J Epidemiol* 1994; **140**: 1111-1124
28 **Cockburn WC**. The work of the WHO Consultative Group on Poliomyelitis Vaccines. *Bull World Health Organ* 1988; **66**: 143-154
29 **Strebel PM**, Ion-Nedelcu N, Baughman AL, Sutter RW, Cochi SL. Intramuscular injections within 30 days of immunization with oral poliovirus vaccine--a risk factor for vaccine-associated paralytic poliomyelitis. *N Engl J Med* 1995; **332**: 500-506
30 **Ion-Nedelcu N**, Strebel PM, Toma F, Biberi-Moroeanu S, Combiescu M, Persu A, Aubert-Combiescu A, Plotkin SA, Sutter RW. Sequential and combined use of inactivated and oral poliovirus vaccines: Dolj District, Romania, 1992-1994. *J Infect Dis* 1997; **175** Suppl 1: S241-S246
31 National Childhood Vaccination Schedules. Available from: URL: http://www.euvac.net/graphics/euvac/vaccination/vaccination.html
32 **Băicuş A**, Persu A, Combiescu M, Aubert-Combiescu A. The maintaining of the active laboratory-based surveillance of the acute flaccid paralysis (AFP) cases in Romania in the framework of the strategic plan of the global polio eradication initiative. *Roum Arch Microbiol Immunol* 2007; **66**: 44-50
33 **Combiescu M**, Guillot S, Persu A, Baicus A, Pitigoi D, Balanant J, Oprisan G, Crainic R, Delpeyroux F, Aubert-Combiescu A. Circulation of a type 1 recombinant vaccine-derived poliovirus strain in a limited area in Romania. *Arch Virol* 2007; **152**: 727-738
34 **Baicus A**, Persu A, Dinu S, Joffret ML, Delpeyroux F, Oprisan G. The frequency and biodiversity of poliovirus and non-polio enterovirus strains isolated from healthy children living in a limited area in Romania. *Arch Virol* 2011; **156**: 701-706
35 **Adu F**, Iber J, Bukbuk D, Gumede N, Yang SJ, Jorba J, Campagnoli R, Sule WF, Yang CF, Burns C, Pallansch M, Harry T, Kew O. Isolation of recombinant type 2 vaccine-derived poliovirus (VDPV) from a Nigerian child. *Virus Res* 2007; **127**: 17-25
36 **WHO**. An update of ongoing research in the Global Polio Eradication Initiative. Poliopipeline 2011. Available from: URL: http://www.polioeradication.org/Portals/0/Document/Research/PolioPipeline/PolioPipeline_08.pdf
37 **Grassly NC**, Wenger J, Durrani S, Bahl S, Deshpande JM, Sutter RW, Heymann DL, Aylward RB. Protective efficacy of a monovalent oral type 1 poliovirus vaccine: a case-control study. *Lancet* 2007; **369**: 1356-1362
38 **Centers for Disease Control and Prevention (CDC)**. Progress toward interruption of wild poliovirus transmission--worldwide, 2008. *MMWR Morb Mortal Wkly Rep* 2009; **58**: 308-312
39 Global Polio Eradication Initiative. Monthly Situation Report (online). Geneva: WHO, 2009. Available from: URL: http://www.polioeradication.org
40 **World Health Organization Country Office Tajikistan**; WHO Regional Office for Europe; European Centre for Dis-

ease Prevention and Control. Outbreak of poliomyelitis in Tajikistan in 2010: risk for importation and impact on polio surveillance in Europe? *Euro Surveill* 2010; **15**: pii=19558

41 **Centers for Disease Control and Prevention (CDC)**. Update on vaccine-derived polioviruses. *MMWR Morb Mortal Wkly Rep* 2006; **55**: 1093-1097

42 **Mohammed AJ**, AlAwaidy S, Bawikar S, Kurup PJ, Elamir E, Shaban MM, Sharif SM, van der Avoort HG, Pallansch MA, Malankar P, Burton A, Sreevatsava M, Sutter RW. Fractional doses of inactivated poliovirus vaccine in Oman. *N Engl J Med* 2010; **362**: 2351-2359

43 **Resik S**, Tejeda A, Lago PM, Diaz M, Carmenates A, Sarmiento L, Alemañi N, Galindo B, Burton A, Friede M, Landaverde M, Sutter RW. Randomized controlled clinical trial of fractional doses of inactivated poliovirus vaccine administered intradermally by needle-free device in Cuba. *J Infect Dis* 2010; **201**: 1344-1352

44 **Baldwin SL**, Fox CB, Pallansch MA, Coler RN, Reed SG, Friede M. Increased potency of an inactivated trivalent polio vaccine with oil-in-water emulsions. *Vaccine* 2011; **29**: 644-649

45 **Kreeftenberg H**, van der Velden T, Kersten G, van der Heuvel N, de Bruijn M. Technology transfer of Sabin-IPV to new developing country markets. *Biologicals* 2006; **34**: 155-158

46 **Simizu B**, Abe S, Yamamoto H, Tano Y, Ota Y, Miyazawa M, Horie H, Satoh K, Wakabayashi K. Development of inactivated poliovirus vaccine derived from Sabin strains. *Biologicals* 2006; **34**: 151-154

47 **Aylward B**, Yamada T. The polio endgame. *N Engl J Med* 2011; **364**: 2273-2275

S- Editor Zhang SS **L- Editor** Roemmele A **E- Editor** Zheng XM

World Journal of Virology

Online Submissions: http://www.wjgnet.com/esps/
wjv@wjgnet.com
doi:10.5501/wjv.v1.i4.115

World J Virol 2012 August 12; 1(4): 115-130
ISSN 2220-3249 (online)

REVIEW

Human T-lymphotropic virus proteins and post-translational modification pathways

Carlo Bidoia

Carlo Bidoia, Centre for Research in Infectious Diseases, School of Medicine and Medical Science, University College Dublin, Belfield, Dublin 4, Ireland
Carlo Bidoia, Department of Surgical and Morphologic Sciences, University of Insubria, Padiglione Biffi via Ottorino Rossi, 9, 21100 Varese, Italy
Author contributions: Bidoia C solely contributed to this paper.
Correspondence to: Carlo Bidoia, PhD, MSc, Department of Surgical and Morphologic Sciences, University of Insubria, Padiglione Biffi via Ottorino Rossi, 9, 21100 Varese, Italy. carlo.bidoia@gmail.com
Telephone: +39-332-217605 Fax: +39-332-217608
Received: August 8, 2011 Revised: June 4, 2012
Accepted: July 13, 2012
Published online: August 12, 2012

Abstract

Cell life from the cell cycle to the signaling transduction and response to stimuli is finely tuned by protein post-translational modifications (PTMs). PTMs alter the conformation, the stability, the localization, and hence the pattern of interactions of the targeted protein. Cell pathways involve the activation of enzymes, like kinases, ligases and transferases, that, once activated, act on many proteins simultaneously, altering the state of the cell and triggering the processes they are involved in. Viruses enter a balanced system and hijack the cell, exploiting the potential of PTMs either to activate viral encoded proteins or to alter cellular pathways, with the ultimate consequence to perpetuate through their replication. Human T-lymphotropic virus type 1 (HTLV-1) is known to be highly oncogenic and associates with adult T-cell leukemia/lymphoma, HTLV-1-associated myelopathy/tropical spastic paraparesis and other inflammatory pathological conditions. HTLV-1 protein activity is controlled by PTMs and, in turn, viral activity is associated with the modulation of cellular pathways based on PTMs. More knowledge is acquired about the PTMs involved in the activation of its proteins, like Tax, Rex, p12, p13, p30, HTLV-I basic leucine zipper factor and Gag. However, more has to be understood at the biochemical level in order to counteract the associated fatal outcomes. This review will focus on known PTMs that directly modify HTLV-1 components and on enzymes whose activity is modulated by viral proteins.

Key words: Human T-Lymphotropic virus; Tax; Rev; p12; p13; Gag; Post-translational modification

Peer reviewer: Barry J Margulies, PhD, Associate Professor, Department of Biological Sciences, Towson University, 8000 York Rd., Towson, MD 21252, United States

Bidoia C. Human T-lymphotropic virus proteins and post-translational modification pathways. *World J Virol* 2012; 1(4): 115-130 Available from: URL: http://www.wjgnet.com/2220-3249/full/v1/i4/115.htm DOI: http://dx.doi.org/10.5501/wjv.v1.i4.115

INTRODUCTION

Infection by human T-lymphotropic virus type 1 (HTLV-1) can cause a fatal hematopoietic malignancy (adult T-cell leukemia/lymphoma, ATLL) and a debilitating neurological disorder (HTLV-1-associated myelopathy/tropical spastic paraparesis, HAM/TSP).

Post-translational modifications (PTMs), like phosphorylation[1,2], ubiquitination[2,3], SUMOylation[4] and others[5], determine the fate of many cellular proteins altering their conformation (charge or hindrance), subcellular localization[6,7], and cell-cycle-related function[8]. PTMs control also gene and viral expression. Such epigenetic control is governed by PTMs on histones, devoted to the organization of chromatin together with the ATP-dependent chromatin remodeling complexes. In particular, LTR silencing is a mechanism used by cells to repress viral transcription. Histone deacetylation and methylation enforce the promoter silencing condensing the chromatin[9].

Recently, Matsuoka and collaborators[10] found that 68 % of fresh ATLL cell lines contained methylated HTLV-1 LTR and 14 % had fully methylated LTR, suggesting a correlation between the development of leukemia, the reduction of Tax expression and genetic (*tax* gene mutations) and epigenetic (LTR histone deacetylation and methylation) modifications. This evidence is coherent with the over-expression of methyltransferase SUV39H1, which silences HTLV-1 LTR, in transformed and latently infected cell lines[11].

In HTLV-1 infected cells the proviral LTR binds Tax, cyclic AMP response element binding protein (CREB), CREB-2, activating transcription factor (ATF)-1, ATF-2, c-Fos, c-Jun (transcription factors), CREB-binding protein (CBP), the histone acetyl transferase (HAT) p300 (both co-activators) and histone deacetylases (HDACs)[12]. The presence of HATs at the viral LTR coincides with histone (H3 and H4) acetylations at three regions within the proviral genome[12]. Viral transcription is subsequent to p300/Tax binding and following p300-dependent histone acetylation on their N-terminal tails[13,14]. Unexpectedly, the use of a HDAC inhibitor, the depsipeptide, in a murine ATLL model inhibits the tumor growth[15]. Also the chromatin remodeler Brahma-related gene 1 is required for Tax transactivation and acts with the PBAF complex for the nucleosome remodeling[16], where the subunit Baf53, suppressor of transcription, may be phosphorylated and hence displaced from the LTR, activating the transcription (as proposed by Kashanchi F. and collaborators[17]).

In addition to the epigenetic control of viral transcription, PTMs regulate viral protein functions by modifying their subcellular localization, stability, and network of interactions[18-20]. Furthermore, viral proteins recruit and alter the function of enzymes responsible for PTMs, like for example ubiquitin ligases and kinases[21-23], altering cellular pathways controlled by PTMs. Gathering all HTLV-1-related reports in a review become more important now, in the light of the growing interest of the scientific community in the field (15th International Conference on Human Retrovirology, HTLV and Related Viruses, Leuven, Belgium, 2011[17]).

HTLV-1 encodes structural and enzymatic viral gene products, the positive regulatory proteins Tax and Rex along with viral accessory proteins such as p12, p13 and p30[24-27]. In addition the virus encodes the HTLV-I basic leucine zipper factor (HBZ) protein within the minus strand[28,29]. These proteins are subjected to PTMs, as any component of the cellular environment, with crosstalk between different PTMs in the same protein[2]. Here, a compendium of the known viral protein PTMs, relevant to the viral function, is presented together with the set of known PTMs directly activated or inhibited by HTLV-1 components. Effects not obviously attributable to single viral proteins are described first.

HTLV-1 INFECTION PROMOTES PTMS

Cerebrospinal fluid in HAM/TSP

The only documented PTM difference in the cerebrospinal fluid between asymptomatic carriers and HAM/TSP patients is enhanced Tau-protein phosphorylation (Tau is an abundant CNS molecule involved in stabilizing microtubules) and an increase in axonal ubiquitination[30]. Valenzuela and collaborators recently found that cyclin-dependent kinase (Cdk)5 prevents Tau hyper-phosphorylation caused by HTLV-1-infected cell secretions[31], but further studies are needed to understand the relevance of those modifications.

Heat shock protein 90, Akt signaling and Lyn

Heat shock protein 90 (Hsp-90), a molecular chaperone, is overexpressed in HTLV-1-infected cells and primary ATLL cells[32] and is hypothesized to be involved in the stabilization of viral proteins. Interestingly, Hsp-90 inhibition, in ATLL cells, induces cell death and promotes de-phosphorylation and inactivation of Akt with subsequent GSK3 activation, which phosphorylates β-catenin for ubiquitination[33]. In turn, ubiquitinated β-catenin is targeted for degradation, probably by β-transducin repeat-containing protein (β-TrCP)[34]. Otherwise, β-catenin enters to the nucleus and forms with T-cell transcription factor an active complex on target genes, such as c-*Myc* and c-*Jun*[33], inducing cell proliferation (Figure 1A).

Collectively these results suggest an involvement of Hsp-90 overexpression in maintaining activation of Akt signaling, hence promoting cell survival[35]. Interestingly, within the setting of B-cell lymphocytic leukemia (BCLL), Hsp-90 stabilizes and activates constitutively Lyn[36], a member of the Src family kinases (SFK) downstream the T cell receptor (TCR) pathway and upstream Akt pathway. Active Lyn appears to determine the activation of Janus family tyrosine kinase 3 (Jak-3) and signal transducer and activator of transcription 5 (Stat-5)[37], important during HTLV-1 infection (see relevant section below). The disruption of the complex containing activated Lyn and Hsp-90 revealed to be a potential pharmacological target for BCLL therapy[36]. This notion could be transposed in the setting of ATLL, considered that Lyn replaces Lck in HTLV-1 infected T cells[37]. It will be of great interest for therapy, to corroborate experimentally the hypothesis that Hsp-90 and Lyn sinergically cooperate at the onset of ATLL to promote T cell activation and proliferation.

TCR signaling

TCR pathway promotes T cell proliferation. Upon TCR engagement, Lck and Fyn phosphorylate immunoreceptor tyrosine-based activation motifs, which act as docking sites for Syk-family kinases, primarily ZAP-70 in T cells. Activated ZAP-70 can in turn phosphorylate the linker for activation of T cells (LAT), thus triggering a number of cascades, culminating in T-cell activation[38].

The signal transduction is not efficient in HTLV-1-infected cells for the progressive decrease in CD3 expression[39]. While CD3 is reduced, the TCR pathway is not inactivated. Recently, authors provided evidences that, in HTLV-1-infected cells, a switch occurs between Lck and Lyn expression, effector typically expressed in B cells[37,40]. Furthermore, FynB and Syk, poorly represented in T

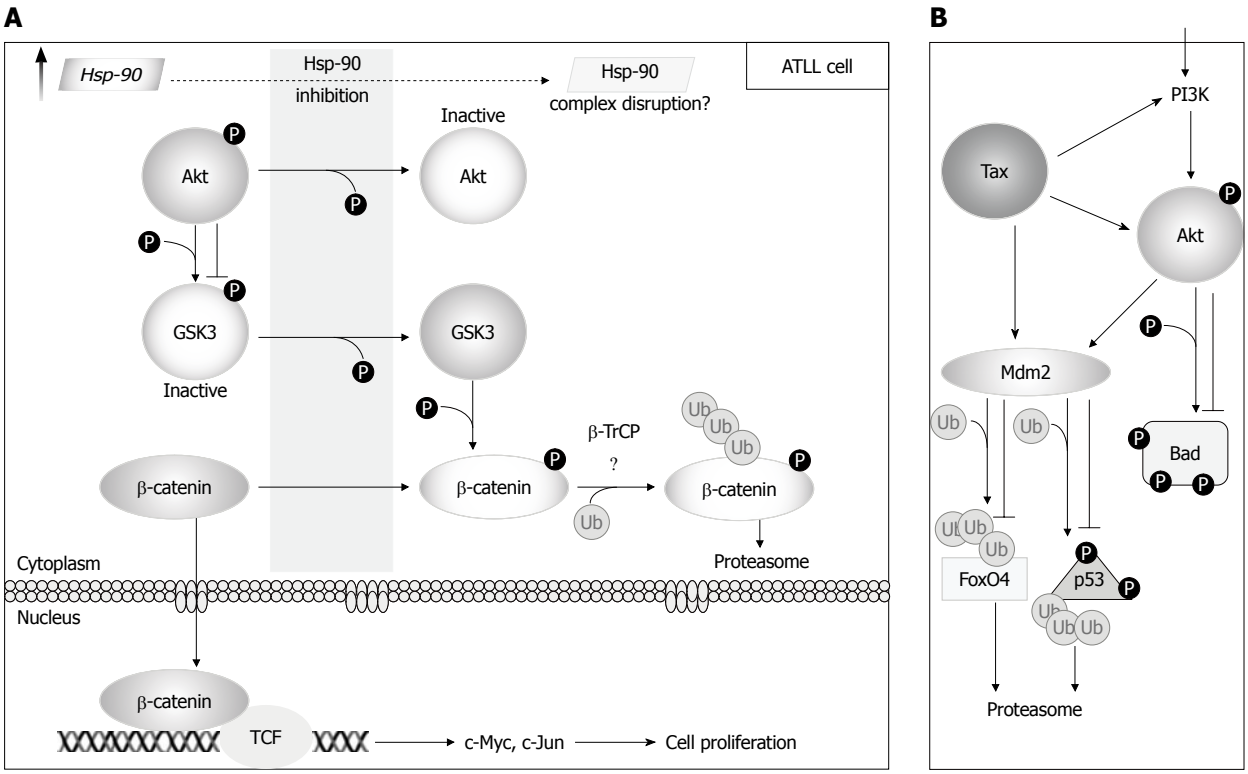


Figure 1 Diagrams representing Akt signaling alterations in adult T-cell leukemia/lymphoma cells under treatment with heat shock protein 90 inhibitors (panel A) and Tax contribution on Akt signaling (panel B). A: In adult T-cell leukemia/lymphoma (ATLL) cells, geldanamycin-derivates inhibition of heat shock protein 90 (Hsp-90) promotes Akt inactivation and hence GSK3 activation for β-catenin degradation, and cell death; B: Activation of Jak/Stat or T cell receptor signaling or Tax expression promote phosphatidylinositol-3-kinase (PI3K) activation, Akt activation and Mdm2 activation with subsequent inactivation of Bad and degradation of forkhead box O4 (FoxO4) and p53. Question marks represent hypothesis; P: Phosphorylation; Ub: Ubiquitin; thick head arrows represent activation, thin head arrows represent enzymatic reactions; dashed arrow, inhibition; protein overexpression in infected cells is represented by bold arrow.

cells, are overexpressed, where ZAP-70 is absent[41]. It is still unclear why the T cell is prompted to express effectors present in B cells, namely Lyn, FynB and Syk, but, intriguingly, it suggests a possible parallelism between ATLL and BCLL.

Downstream ZAP-70/Syk and LAT, activated Ras triggers many Ser/Thr kinases, including ERK, JNK and MAPKp38[42], members of the mitogen-activated protein kinase family (MAPK). MAPK are activated in response to DNA damage[43] and decide the fate of the cell, promoting either repair of the damage or, if the damage is too severe to be repaired, apoptosis. Yamaoka and collaborators recently showed that protein geranylgeranylation is responsible for ATLL cells viability[44]. This contribution of the geranylgeranylation to the ATLL cell survival could be correlated with PTM geranylgeranylation of Ras, a necessary step for its translocation to the plasma membrane, where it is functionally active[45]. In Tax-expressing cells, Tax modulates and increases the phosphorylation (activation) of Ras and ERK kinases, thus facilitating cell proliferation[46]. In this context, Tax protects from apoptosis cooperating with Ras in activating ERK signaling[47]. Furthermore, Tax physically interacts, through its PDZ binding motif, and blocks the function of Erbin, negative regulator of the Ras-Raf-ERK pathway, enhancing the cell proliferation and the protection from apoptosis[48].

Collectively, these results indicate that during HTLV-1 infection TCR response to stimuli is hampered and, at the same time, downstream effectors not belonging to T cells are upregulated, promoting constitutive activation of T cells. Interestingly, p12, p13, p30 and Tax possess putative SH3 binding motifs, whose possible interaction with SFK is not fully explored. Further studies on viral TCR perturbation could guarantee access to new therapeutic approaches.

Jak/Stat

Jak/Stat activation appears to be associated with the replication of primary ATLL cells[49]. In particular, it has been previously shown in patients that more than 65% of T cells possess constitutive DNA-binding activity of one or more Stat proteins and this activity seems to persist over time[49].

Interleukin (IL)-2 stimuli rapidly induce tyrosine phosphorylation of intracellular substrates, including its receptor IL-2Rβ, Jak-1 and -3 and Stat elements[50]. HTLV-1-immortalized cell lines often exhibit constitutive Jak/Stat phosphorylation and activation[51], differently from HTLV-2-infected cells[52]. In T cells immortalized with a molecular clone of HTLV-1, the Jak/Stat signaling during IL-2 stimulation seems to promote HTLV-1 transmission[53].

Interestingly, in a rabbit HTLV-1-infected cell line, bearing truncated forms of p13 and p30 (RH/K34, see

relevant section below), the Jak kinase Tyk2 and Stat3 are constitutively phosphorylated and activated[54]. Surprisingly, in other cell lines, HTLV-1-infected 293T and HeLa cells, phosphorylation and activation of Tyk2 and of Stat2 are reduced, for the reduction of the IFNα signal transduction[55]. In human T cells, inhibition of Jak2/Jak3 does not seem to block the proliferation of HTLV-1-transformed and IL-2-independent cells, Hut102B2 and MT-2[56], since the Jak/Stat pathway seems functionally redundant for proliferation. In contrast with that finding, Waldmann and collaborators recently demonstrated that CP-690,550, a specific inhibitor of Jak3, suppresses proliferation of peripheral blood mononuclear cells (PBMCs) from ATLL and HAM/TSP patients, promoting the drug as an effective therapeutic agent in patients whose cells require Jak3/Stat5 stimulation by γc cytokines (IL-2, IL-9, and IL-15) to proliferate[57].

In turn, the suppressor of cytokine signaling (SOCS), inhibitor of Jak/Stat signaling, is upregulated in HTLV-1$^+$ CD4$^+$ T cells from HAM/TSP and asymptomatic carriers, and correlates with viral replication[58]. In particular, SOCS-1 inhibits IFN expression and IFN-stimulated gene expression[58]. SOCS adaptors are bound to cullin-2 and -5 through elongin C and elongin B proteins to constitute ubiquitin ligase complexes[59]. At the 15th International Conference, Harhaj E. W. and collaborators showed that, in the presence of Tax, the expression and the stability of SOCS-1 are highly increased[17]. The independence from cytokines in infected cells could be associated to the inhibition of the upstream effectors through a mechanism requiring ubiquitination that should be further investigated.

Cell cycle

The cell cycle is controlled by a cyclic series of activation and repression though phosphorylation cascades and is profoundly altered during HTLV-1 infection[60]. HTLV-1-infected, as well as ATLL and HAM/TSP cells, exhibit overexpression of the Cdk inhibitor p21/Waf1[61]. p21/Waf1 interacts specifically with the Cdk2/cyclin A complex and reduces the phosphorylation level of the retinoblastoma protein (Rb), a tumor suppressor, and prevents the release of E2F-1, transcription factor required for G1 and S phases[62].

p27/Kip1 is an other Cdk inhibitor and blocks the Cdk2/cyclin E complex. p27/Kip1 upregulation arrests the cell cycle in G1 upon serum starvation[63]. Interestingly, HTLV-1-transformed cells exhibit a higher level of the p27/Kip1 than the HTLV-1-immortalized cells[63].

Additionally, Aurora kinases are overexpressed in HTLV-1 positive cells. Aurora kinases play an important role in cell cycle control, since their inhibition arrests mitosis and serves to induce apoptosis[64]. Aurora A is overexpressed in HTLV-1-infected and ATLL cells. siRNA knockdown experiments and chemical inhibition of Aurora A has been reported to lead to growth suppression in HTLV-1-infected cell[65]. The ubiquitin ligase CHFR regulates the turnover of Aurora A, and in infected cells CHFR level is reduced by methylation and subsequent silencing of its promoter[65]. Aurora B is overexpressed in HTLV-1-infected cells and its specific inhibition induces apoptosis only in HTLV-1-infected cells compared to non-infected, through overexpression of p21/Waf1 and p53[66].

The overexpression of Cdk inhibitors is concurrent with the upregulation of Aurora kinases, with the p30-related delay of entry into S phase and the Tax-related delay of S-G2-M transition (Figure 2). The contrasting results on the cell cycle alterations attributed to HTLV-1 are far from being understood and evaluated for therapy purposes.

HTLV-1 P40, THE TRANS ACTIVATOR OF TRANSCRIPTION TAX

Several pathways are activated or repressed after HTLV-1 infection and single viral proteins act to mediate with specific PTMs and interactions these downstream effects. The most important is the transactivator of transcription, Tax. In Figure 3 is represented a model for Tax localization depending on its PTMs.

Tax is a pleiotropic protein with a predominately nuclear subcellular localization that performs multiple functions *via* protein-protein interaction. Several Tax PTMs were described, including ubiquitination[67], SUMOylation[67], phosphorylation[68-70] and acetylation[71]. Probably, different Tax species coexist at any time during the cell cycle and in different locations, depending on the cell type (see for example its ubiquitination state in[72]).

Recently, Kfoury *et al*[67] showed by live confocal microscopy, that Tax molecules shuttle from SUMO-enriched nuclear bodies to ubiquitin-enriched perinuclear centrosomal aggregates close to *cis*-Golgi (Figure 3). Those molecules are likely PTM Tax variants, considered that the SUMOylation and ubiquitination-defective Tax K4-8R mutant is not recruited to these subcellular compartments[67].

Biochemical coupling of the transcription factor nuclear factor κB (NF-κB) to antigen and co-stimulatory receptors (CD3 and CD28, respectively) is required for the temporal control of T-cell proliferation and is modulated by viral proteins[38]. Tax activates NF-κB by interacting and constitutively stimulating the activity of the I-κB kinase (IKK)[73], which usually is transiently activated through the TCR/CD3 and CD28 engagement. Tax SUMOylation sends the IKKγ (known as NEMO) to the centrosome[67] and is involved in the formation of Tax-related SUMO-enriched nuclear bodies and in the recruitment therein of RelA (known as p65), resulting in transcriptional activation[74,75]. On the contrary, ubiquitin addition appears to modify Tax in a proteasome-independent manner from an active to a less-active transcriptional form[76], even though Tax ubiquitination is critical for the relocalization of IKK complexes to perinuclear hot spots coinciding with Golgi[77]. Counterintuitively, Nasr *et al*[74] have shown that Tax ubiquitination and SUMOylation are dispens-

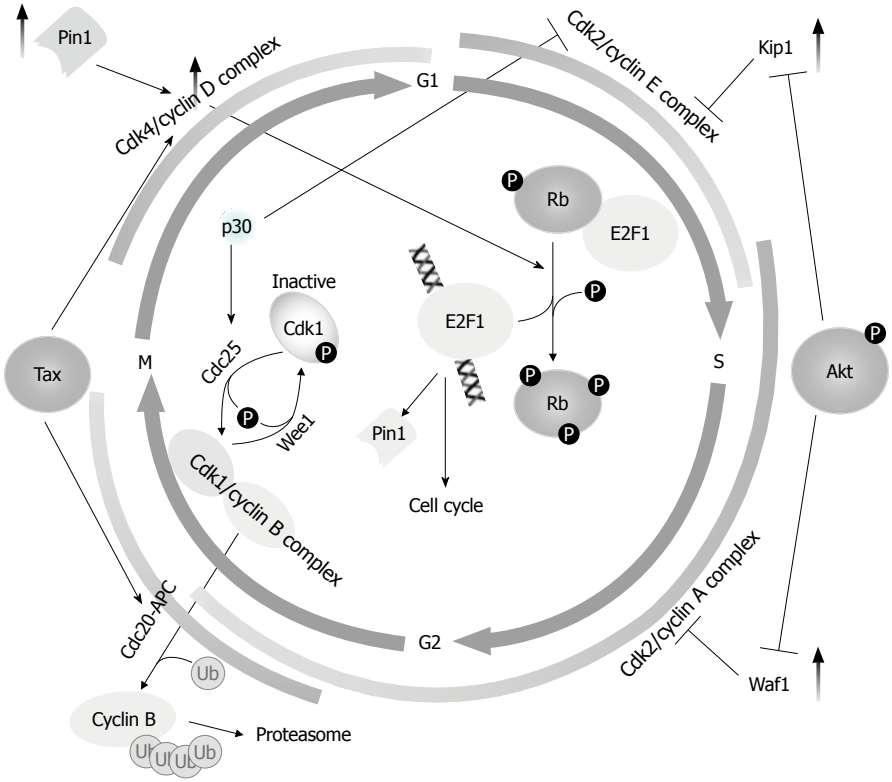


Figure 2 Cell cycle deregulation in infected cells and Tax-expressing cells. G1 progression begins with cyclin D expression, formation of the Ckd4/cyclin D complex that in turn phosphorylates Rb, inducting E2F1 transcription. Cyclin D is upregulated by Tax and by Ras-Raf-ERK stimulation. It is stabilized by interaction with Pin1, overexpressed in infected cells. G1/S phase transition, promoted by the Ckd2/cylclin E complex is antagonized by p30 and by overexpressed p27/Kip1. Overexpressed p21/Waf1 inhibits Ckd2/cyclin A-promoted S/G2 transition. In turn, overactivation of Akt inhibits p27/Kip1 and p21/Waf1 functions. Tax promotes, through Cdc20-APC activation, cyclin B degradation. At the same level acts p30, that activates Ckd1 by Cdc25-dependent de-phosphorylation. Cycling stability of CKd/cyclin complexes are represented with fading arcs; P: Phosphorylation; Ub: Ubiquitin; thick head arrows represent activation, thin head arrows represent enzymatic reactions; protein overexpression in infected cells is represented by bold arrows.

able for its transcriptional activation of CREB-dependent genes. Indeed, Tax mutants for Lys-85 or Lys-88, poorly contributing to Tax ubiquitination[72], are unable to recruit CBP/p300[78].

Tax ubiquitination

Only recently, it was demonstrated that Tax ubiquitination contributes to viral transcriptional activity, as a prerequisite to recruit IKKγ in the centrosome (known as MTOC, microtubule-organizing center) and in SUMO-enriched nuclear bodies[67] (Figure 3). Ubiquitination is also involved in Tax binding with IKK complex[79] and the upstream transforming growth factor-β-activated kinase 1 (TAK-1) binding protein 2 (TAB2[80]). The authors showed also that overexpression of TRAFs strongly induces Tax ubiquitination[80] (TNF receptor-associated factors, adapter proteins that mediate cytokine signaling[81]).

Two forms of ubiquitinated Tax coexist. The cytoplasmic (Lys-63-branched and Lys-48-branched poly-ubiquitinated, K63 and K48) Tax is stable, as its amount is only modestly increased by proteasome inhibition. The nuclear K48 poly-ubiquitinated Tax is not stable and only under proteasome inhibition is stabilized[82]. Ubiquitin-mediated proteolysis is highly selective and tightly regulated. The specificity of the process relies on the ubiquitin-protein ligases, which directly bind both the substrate and the ubiquitin-conjugating enzymes[59]. Tax K48 poly-ubiquitination involves PDLIM2, an ubiquitin E3 ligase with PDZ and LIM domains[82]. PDLIM2, without the involvement of Tax PDZ binding motif, binds to and recruits Tax from both the cytoplasm and the Tax-related nuclear bodies into the nuclear matrix where the poly-ubiquitinated Tax is degraded by the proteasome[82] (Figure 3). Interestingly, Xiao G. and collaborators have shown that PDLIM2 expression is epigenetically down regulated in primary ATLL cells[17].

Furthermore, it was demonstrated that Tax is mainly K63 poly-ubiquitinated and that this ubiquitination depends on the interaction with the ubiquitin-conjugating enzyme Ubc13, which proved critical for interaction with IKKγ and canonical and non canonical NF-κB activation[83] (Figure 3). Also the NEMO-related protein Optineurin has been shown to interact with ubiquitinated Tax in HTVL-1-infected cells and to promote with Tax1BP1 the NF-κB activation[84]. Interestingly, the Tax mutant M22 is deficient in NF-κB activation, is less ubiquitinated and does not interact with Ubc13[72,83]. Consistently with this results, the ubiquitin-specific peptidase USP20 inhibits Tax transactivation activity, promoting its de-ubiquitination[85] (Figure 3).

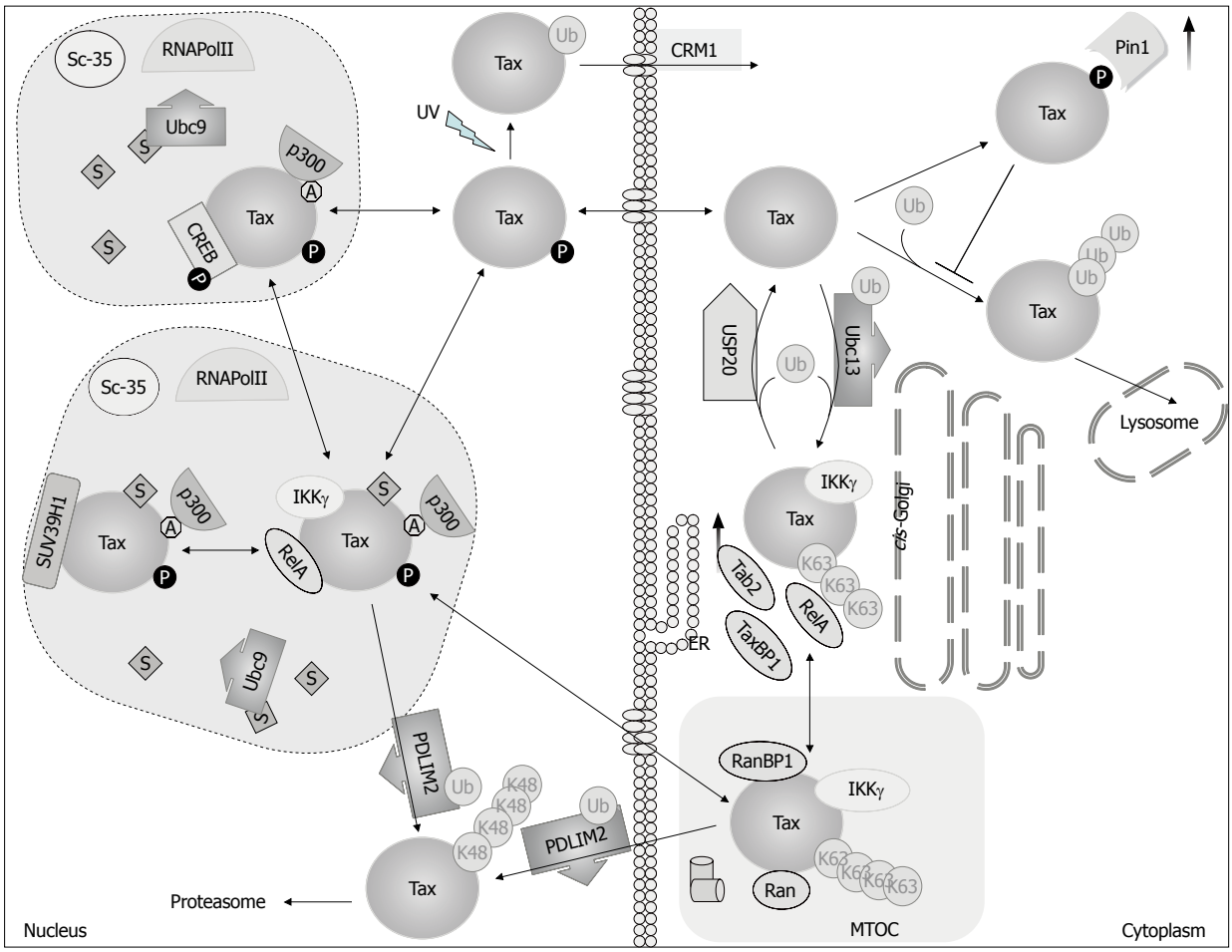


Figure 3 Diagram representing Tax localization and function controlled by post-translational modifications. After translation, Tax could be phosphorylated at Ser-160 and hence stabilized by Pin1, preventing its polyubiquitination with subsequent lysosomal degradation (top right). Tax is K-63 polyubiqitinated by Ubc13, promoting its relocation to a region between endoplasmic reticulum and cis-Golgi, where it interacts with I-κB kinase (IKK)γ, the overexpressed Tab2 and RelA. UPS20 can de-ubiquitinate Tax (centre). From cis-Golgi, Tax shuttles to microtubule-organizing center (MTOC), where it interacts with IKKγ, RanBP1 and Ran. From MTOC, Tax shuttles to Tax-related nuclear bodies containing Sc-35 and RNAPolII. There, it is acetylated by p300 and SUMOylated, probably by Ubc9 (left). In nuclear bodies, Tax interacts with p300, IKKγ, RelA and the methyltransferase SUV39H1. Furthermore, Tax interacts also with phosphorylated cyclic AMP response element binding protein. PDLIM2 has proven to cause K48 polyubiquitination of Tax, promoting its nuclear proteosomal degradation after translocation from nuclear bodies and MTOC (bottom left). Tax displays its nuclear functions when Ser-300 19 or Ser-301 are phosphorylated. Finally, after UV treatment, monoubiquitinated Tax is exported to the cytoplasm through CRM1 (top). Arrows represent relocation or modification; P: Phosphorylation; S: SUMO1; A: Acetylation; Ub: Ubiquitin; K48 or K63: Chains of ubiquitin; UV: Ultraviolet light exposure; protein overexpression in infected cells is represented by bold arrows.

Additionally, UV irradiation has been demonstrated to induce Tax mono-ubiquitination at the SUMOylatable Lys-280 and Lys-284 residues, which promotes Tax dissociation from nuclear bodies and Tax nuclear export through the CRM1 pathway[86] (Figure 3).

Tax phosphorylation

Interestingly, Ser160-phosphorylated Tax is stabilized by the interaction with Pin1, a prolyl isomerase, which inhibits Tax ubiquitination and its subsequent lysosomal degradation[87,88]. Ser-160 phosphorylation happens during the mitosis, but the kinase involved is presently unknown. Remarkably, Pin1 stabilizes other phosphorylated proteins: c-Jun and c-Fos, enhancing the activator protein 1 (AP-1) transcriptional activity; β-catenin, enhancing cell proliferation; RelA, enhancing the NF-κB activity; and cyclin D1, whose transcription is promoted by the three mentioned transcription factors. In turn, Pin1-stabilized cyclin D1 is retained in the nucleus and promotes G1-S transition[89]. Pin1 overexpression disrupts the tight coupling of centrosome duplication with DNA synthesis, which leads to centrosome amplification, chromosome instability (aneuploidy) and oncogenesis both *in vitro* and *in vivo*[90]. The overexpression of Pin1 in Tax-expressing HTLV-1-transformed cells[88] is consistent with previous characterizations showing that Pin1 expression is tighly regulated and correlates with oncogenesis[89]. Finally, Tax with CREB physically associates the promoter of cyclin D1 *in vivo*, upregulates the gene and promotes G1-S transition[91]. On the other hand, Tax delays the S-G2-M transitions, favoring the degradation of cyclin A, cyclin B1 and securin. Cyclin B1 is targeted for ubiquitination by Tax-activated cdc20-associated anaphase promoting complex (cdc20-APC), an E3 ubiquitin ligase that controls metaphase to anaphase transition and, bound to Tax, becomes active before the cellular entry into mi-

tosis[92] (Figure 2). The authors proposed that aneuploidy and chromosomal instability are direct consequences of Tax activation of cdc20-APC. Thus, we can hypothesize that Pin1 and Tax work in a synergistic manner to promote tumorigenesis, path not explored for therapeutic purposes yet.

Tax is known to be phosphorilated since 1988[93]. Research attributed the serine residues were the target of this PTM[94] and useful studies on serine point mutations were performed providing evidence to support this idea[95,96]. Disappointingly, specific Tax phosphorylation patterns are associated with the particular cell line employed[97]. At the present, important phosphorylation sites of Tax are known but still some open question remains. Phosphorylation on Tax Ser-300/301 (or on Ser-304 where present) is essential for the transcriptional activation[68]. Phosphorylation on Ser-160 stabilizes Tax during the mitosis and enhances the activation of NF-κB[87]. The kinases involved are unknown and could represent a genuine target for Tax-oriented therapy. Only the pleiotropic and constitutively active CK2 (formerly known as casein kinase 2) is proven to phosphorylate Tax *in vitro*, modulating the functions associated with its C-terminus[70], which is extremely important for the binding, with subsequent inhibition, of class I PDZ-containing proteins, some of them tumor suppressors with scaffold function[98,99].

Furthermore, advanced and highly sensitive techniques, such as tandem mass spectrometry, struggle to detect Ser-300/301 phosphorylation or are entirely unable to detect Ser-160 phosphorylation, important for Tax function[69]. The coverage of N- and C-terminal tagged Tax is incomplete for technical limitations and, surprisingly, threonine phosphorylation predominates. The phosphorylated residues within Tax described to date are Thr-48, Thr-184, Thr-215, Ser-300 or 301 and Ser-336[69]. Interestingly, in functional studies, the authors showed that a phosphomimetic point mutation on the Thr-48, always found phosphorylated, reduced Tax activity on CREB and NF-κB promoters, as if Tax function was always switched off. Mutation of the predominantly phosphorylated residue Thr-215 to the phosphomimetic aspartate led to a severe reduction of Tax activity[69], suggesting again the predominant presence of inactive Tax. Lastly, discordance between peptide identification (Thr-84 peptide) and Tax sequence in Durkin's report generates ambiguity and makes it difficult to draw neat conclusions. Point mutations do not seem to clearly delineate a conclusive definition of the roles of phosphorylated Tax residues.

Due to the complexity of the Tax phosphorylation pattern, the study of multiple point mutations (all-but-one) and mutant expression in lymphocytes could contribute to the comprehension of the actual relevance of each phosphorylation for Tax function. Also the identification of kinases involved in Tax phosphorylation could suggest new therapeutical strategies.

Tax acetylation and SUMOylation

Tax acetylation on Lys-346 is dependent on Tax localization in Tax-related nuclear bodies and on its interaction with the transcriptional co-activator p300[71] and subsequent activation of HTLV-1 promoter[13]. The prerequisite of nuclear Tax localization is phosphorylation on Ser300/301 (or on Ser-304 where present[68]). Its localization on Tax-related SUMO-enriched nuclear bodies is followed by SUMOylation (for the presence of the residues Lys-280 and Lys-284[71]) that, in turn, improves Tax acetylation. When SUMOylated Tax is acetylated by p300, its activity on integrated NF-κB promoter is enhanced[71]. In SUMO-enriched nuclear bodies, Tax colocalizes with Ubc9, an E2-SUMO conjugating enzyme with a major nuclear localization and, when ubiquitinated, promotes SUMOylation and de-ubiquitination of IKKγ[67]. One appealing highlighted hypothesis is that Tax is SUMOylated in those Ubc9-enriched bodies.

Tax-2B PTMs

A recent review described the known HTVL-1/2B Tax differences in NF-κB activation and localization, and showed how PTMs affect the two homologue proteins that share 85 % of sequence similarity[99]. HTLV-2B Tax is SUMOylated at lower levels than HTLV-1 Tax and ubiquitinated[100]. Intriguingly, Mahieux R. and collaborators showed at the 15th International Conference that ubiquitinated HTLV-2 Tax is barely detectable but still active on NF-κB pathway, promoting IKKγ and RelA nuclear relocalization[17].

Tax and NF-κB PTMs

The NF-κB pathway is correlated with the oncogenic potential of Tax. IKK activation is involved in both the canonical and non-canonical NF-κB pathways, and one of the main differences between HTLV-1 Tax and the less oncogenic HTLV-2 Tax is the ability to process p100, component of the non-canonical pathway[99]. In HTLV-1-infected T cells, p100 is actively processed, resulting in Tax-mediated p52 overproduction and NF-κB2 activation. Tax acts on p100 indirectly recruiting the IKK complex and directly binding p100, IKKα, IKKγ and the β-TrCP[59]. β-TrCP is an F-box adaptor protein, a component of the ubiquitin ligase SCF (Skp-1, Cullin-1, F-box protein), it determines the substrate specificity for ubiquitination[59] and is involved in Tax-mediated p100 processing. NIK-phosphorylated IKKα and Tax-activated IKKγ phosphorylate p100[101]. Tax increases the interaction between the adaptor β-TrCP and IKKα-phosphorylated p100, favoring p100 Cullin-1-mediated poly-ubiquitination and degradation to the active form p52[102]. Interestingly, β-TrCP is probably associated with the degradation of β-catenin[34], a protein stabilized by Pin1[89], and I-κBα[103].

In Tax-expressing cells, IKKβ is persistently phosphorylated, promoting I-κBα degradation and NF-κB activation[104], and subsequently mono-ubiquitinated by the ubiquitin ligase Ro52, in cooperation with the ubiquitin-conjugating enzyme UbcH5B[105,106]. Mono-ubiquitination redirects IKKβ to the autophagosome[106]. Moreover, Tax

interacts physically with the hypo-phosphorylated NF-κB inhibitor I-κBα, promoting its direct binding to HsN3 proteasome for degradation[107].

It is unclear whether the interaction between the TAK-1 and Tax promotes the phosphorylation and activation of IKKβ[108] or not[109]. Tax activates TAK-1 in HTLV-1-transformed T cells. Tax induces also TAB2 overexpression, a TAK-1 activator[109]. Interestingly, both HTLV-1 and -2B Tax interacts with TAB2 in perinuclear compartments close to *cis*-Golgi[110] (Figure 3). TAK-1 activity seems to be involved in JNK and MAPKp38 activation, the former to activate the ATF-2, the latter to inhibit TAK-1 in a negative loop[109]. Furthermore, during IL-2 deprivation, the viral induction promotes phosphorylation and activation of MAPKp38, in turn activating its downstream CREB and sustaining HTLV transcription in HTLV-1-infected cell[111].

Another candidate for IKK activation is the Tax interactor, MAPK kinase kinase 1 (MAP3K1, known also as MEKK1), a kinase that activates JNK and MAPKp38. MAP3K1 is part of the IKK complex, interacts with Tax and seems to activate IKK itself[104].

There are no doubts that NF-κB pathway is required for the activation and proliferation of infected cells, even though evidences suggest an inhibitory effect of HBZ on the canonical pathway[112]. The activation of the non-canonical pathway should be further explored, considered it is one of the main differences between Tax-1 and its homologue proteins, Tax-2B[99].

Tax and Akt PTMs

A growing list of ubiquitin ligases recruited by Tax has been described. An example is Mdm2 ubiquitin ligase. The forkhead box O4 (FoxO4), a transcription factor and tumor suppressor situated downstream Akt signaling, is degraded after activation, for Tax-mediated interaction with Mdm2 and subsequent poly-ubiquitination[113].

Mdm2 is also implicated in the degradation of the phosphorylated and hence inactivated p53 tumor suppressor[114,115]. p53 is hyper-phosphorylated in HTLV-1-transformed cells and cannot interact with the transcription factor IID. Tax promotes p53 phosphorylation and inactivation, but it is unclear whether through RelA engagement[116] or through Tax/p53 competition for binding with CBP[117,118]. Also HTLV-2B Tax, but not HTLV-2A Tax, is able to neutralize p53 activity[119] (Figure 1B).

p53 inhibition and cell survival in HTLV-1-transformed cells are linked to the Tax activation of Akt[39] and phosphatidylinositol-3-kinase (PI3K)[120]. Active Akt promotes phosphorylation and inactivation of Bcl-2-associated death promoter protein (Bad), a pro-apoptotic protein[39]. Consistently, PI3K/Akt activation induces resistance to IL-2 deprivation in HTLV-1-infected cells, promoting a progressive resistance to apoptosis[121].

Tax and promoter silencing

Tax/CREB complex recruits the co-activators CBP and p300, promoting the histone acetylation and disassembly of the nucleosomes from the HTLV-1 promoter, facilitating DNA accessibility for the transcriptional machinery. The process is transcription- and ATP- independent, but needs acetyl-CoA and available histone-chaperone Nap1[122,123].

On the other hand, Tax and active HDAC1 form an inhibitory complex on the promoter of Src homology-2 containing protein tyrosine phosphatase 1 (SHP-1), an important negative regulator of the TCR signaling for its ability to antagonize Src kinases[124] and IL-2 signaling[125]. Remarkably, SHP-1 is inactivated in HTLV-1-immortalized (IL-2-independent) cell lines[125] but not in ATLL cells where Tax is not detectable[126]. Not surprisingly, also the TCR effector ZAP-70 is down regulated by Tax expression[41].

Moreover, Tax forms complexes also with both HDAC1 and HDAC3, promoting a negative feedback loop in which Tax transcription is down-regulated through histone deacetylation on the HTLV-1 LTR region[127]. Watanabe T. and collaborators proposed an other negative feedback and demonstrated that Tax induces the histone methyltransferase SUV39H1 expression[11]. Tax tethers the enzyme to the Tax-related nuclear bodies where it represses Tax, in a dose-dependent manner, silencing the HTLV-1 LTR promoter[11].

Unexpectedly, the use of a HDAC inhibitor, the depsipeptide, in a murine ATLL model inhibits the tumor growth[15], instead of promoting it, probably for the intervention of the adaptive immunity on the desilenced cells.

Furthermore, HTLV-1 transcription increases after CD2 cross-linking and T cell activation[128]. The CD2 signaling promotes CREB phosphorylation probably with the involvement of both protein tyrosine kinases and protein kinase A (PKA)[129], thereby resulting in promoting transcription. In agreement with this mechanism, it seems that Tax promotes CREB phosphorylation and interacts with phosphorylated CREB[130]. Remarkably, CREB-2 cannot be phosphorylated by PKA and is still able to interact with Tax to promote HTLV-1 transcription[131].

Tax hampers DNA damage response

In response to DNA damage, Chk2 protein kinase is phosphorylated and activated, and produces cell cycle arrest. Chk2 and DNA-dependent protein kinase form a complex with Tax in nuclear bodies[132]. Tax induces constitutive activation of this complex and hampers the response to new damage, suggesting a saturation of the DNA repair pathway caused by Tax[132]. Tax oncogenic protein exploits and manipulates many cellular pathways involving PTMs. The picture is far from complete, due to the diverse Tax performances (and variants) among different cell lines and cell cycle steps.

HTLV-1 REX PHOSPHORYLATION AND TCR PATHWAY

The phosphoprotein Rex is found predominantly in the cell nucleolus and is involved in splicing and nuclear ex-

port of viral mRNA. Rex recognizes a specific response element on the incompletely spliced mRNAs, stabilizing them and inhibiting their splicing, and utilizes the CRM1 pathway for nuclear export[133], the same used by mono-ubiquitinated Tax[86].

The major targets of Rex phosphorylation in HTLV-1-infected cells are serines and at a lesser extent threonines[134]. Rex phosphorylation at Ser-70 seems involved in the regulation of Rex function in response to extracellular stimuli, through protein kinase C activation[134]. Decreased Rex phosphorylation correlates with the reduction of unspliced viral mRNA[134].

Recently, Kesic *et al*[135] completely mapped the phosphorylation sites of Rex and found seven phosphorylation sites. Phosphorylations on Ser-97 and Thr-174 were demonstrated to be critical for Rex-1 function[135]. The authors mapped also HTLV-2 Rex and found that phosphorylations on Thr-164, Ser-151 and Ser-153 are functional[136]. In particular, Ser-151 phosphorylation correlates with Rex nuclear/nucleolar localization[136] and with an active Rex protein[137].

For unknown reasons, Rex expression promotes the expression of the Src kinase FynB, usually absent in T cells, thus altering the TCR pathway[41]. This switch to a SFK expressed preferentially in B cells suggests a putative activation of a B cell receptor (BCR) cascade within a T cell, raising new questions and requiring further studies.

HTLV-1 P12, TCR SIGNALING AND PTMS

Endoplasmic reticulum (ER)-localized p12 enhances p300 expression in T cells in a calcium-dependent, but calcineurin-independent manner[138], thereby negating the p30-mediated repression of LTR transcription (see below[139]).

p12 does not seem contribute to the Jak/Stat phosphorylation and activation in HTLV-1-immortalized cells[140], but promotes the phosphorylation of Stat-5 in p12-transduced PBMCs[141]. However, p12 acts downstream of TCR signaling. On one hand, p12 increases cytoplasmic calcium levels inducing the nuclear factor of activated T cells (NFAT) activation in a LAT-independent manner[142]. On the other hand, p12 inhibits the phosphorylation of LAT, Vav, and phospholipase C-γ1 upon engagement of CD3, leading to decreased TCR-induced activation of NFAT[143].

To complicate the picture, two forms of 8 and 12 KDa coexist and can be palmitoylated, as shown at the 15th International Conference by Franchini G. and collaborators. Notably, palmitoylation increases the hydrophobicity of proteins and targets them to lipid rafts[17]. Post-translational removal of the ER retention/retrieval signal located at the amino terminus of p12 yields the p8 protein, which traffics to the cell surface, promoting T cell anergy and clustering, structuring the virological synapse, ultimately increasing the virus spreading[144].

HTLV-1 P13 INTERACTS WITH SFKS

Signal transduction is based on activation of normally inactive kinases that, under external (or internal) stimuli, phosphorylate their substrates often promoting a cascade[145]. The TCR signaling uses the SFKs to transduce the signal[146]. SFKs localize in their inactive state on the inner leaflet of the plasma membrane, promptly transduce the TCR signal following recognition of immunocomplexes on antigen-presenting cells, and ultimately lead to T-cell proliferation and immune response[38].

Interestingly, p13, a mitochondrial and nuclear protein[147], binds the SH3 domain of SFKs[146]. p13/SFK forms a stable heterodimer that translocates to mitochondria by virtue of a p13 N-terminal mitochondrial localization signal[146]. As a result, the activity of SFKs is dramatically enhanced with a concomitant increase in mitochondrial tyrosine phosphorylation[146]. Contrasting reports exist as to whether p13 is able to target the inner mitochondrial membrane and to perturb the mitochondrial membrane potential[146,148].

Further investigations are needed to understand the significance of the potential SFK activation within HTLV-1-infected T cells, considered their importance in the pathogenesis of diseases such as chronic myeloid leukemia and BCLL[149].

These results appear in contrast with the recent findings that p13, in spite of its mitochondrial localization when expressed alone, in the presence of Tax is partially mono-ubiquitinated, stabilized, and re-routed to the Tax-related nuclear bodies[147]. p13 directly binds Tax, decreases Tax binding to the CBP/p300 transcriptional co-activators and, by reducing Tax transcriptional activity in a dose-dependent manner, thereby suppresses viral gene expression[147].

Interestingly, a truncated form of p13 (and hence a truncated p30), lacking of the C-terminal SH3-binding motif, is encoded by the rabbit HTLV-1-infected cell line RH/K34, a renowned cell line for its ability to induce a lethal leukemic-like disease in animal[150]. In contrast with the cell line RH/K30, that produce a low-grade leukemia with acute rejection, a different pattern of tyrosine phosphorylation is noticeable within RH/K34 cells[151]. Remarkably, Vav, a downstream TCR effector, is constitutively phosphorylated and activated only in the RH/K34 cell line. Clearly, while the SH3-binding motif of p13 is not contributing to the constitutive activation of TCR signaling[151], other viral proteins are involved through unidentified mechanisms.

Further functional studies on infected cells and during viral protein co-expression are required to delineate the complex interplay between the viral proteins and the cell, although the presence of putative SH3-binding motifs in p12, p30 and Tax suggests a focused molecular study on their putative physical interaction with SFKs.

HTLV-1 P30 ALTERS CELL CYCLE AND REPRESSES TAX FUNCTION

p30 is a viral accessory protein that localizes with p300 in the nucleus. p30 competes with Tax for the same p300

N-terminal region of binding, eventually repressing Tax transcriptional activity in a dose-dependent manner[139]. Increased availability of ectopically expressed HAT-active p300 reverses the effect of p30. The authors proposed that the intrinsic HAT activity of p300 is utilized to acetylate and potentially modulates the transcriptional regulatory function of p30, but further studies are needed[139].

p30 delays the entry into S phase, decreasing the phosphorylation levels of Rb, while its homologue, HTLV-2 p28, does not. In order to do so, p30 interacts with Cyclin E and with Cdk2 and prevents their activation[152] (Figure 2). p30 also seems to alter the pattern of phosphorylation of the Cdc25C phosphatase, Chk1 checkpoint kinase and Polo-like kinase-1, thereby activating the cell cycle checkpoint at the G2-M phase transition[153] (Figure 2). These data, taken together, suggest a Tax-counteracting effect of p30 on the cell cycle control (as described above), but do not allow a clear characterization of p30 function.

HBZ UTILIZES UBIQUITIN PATHWAYS

HBZ has been shown to negatively regulate basal and Tax-dependent LTR transcription through its ability to interact with specific basic-leucine zipper proteins and to inhibit CREB binding to the LTR[154]. Furthermore, HBZ, like p30, inhibits Tax transcriptional activity competing with Tax for p300 binding[155].

Interestingly, HBZ acts also indirectly on Tax canonical NF-κB transcription, inducing the overexpression of PDLIM2 ubiquitin ligase and interacting with RelA[112]. The authors found that PDLIM2 targets specifically RelA for degradation and that HBZ binds RelA diminishing its DNA binding capacity[112]. Also Tax stability is affected by PDLIM2 as described above. PDLIM2 down regulation in primary ATLL cells[17] suggest a balanced interplay between HBZ and other viral proteins in which HBZ activity is restricted.

Remarkably, HBZ promotes c-Jun degradation without the requirement for ubiquitination[156], counteracting its stabilization promoted by Pin1 (described above). HBZ appears to function as a tethering factor between the 26S proteasome and c-Jun, thus repressing the AP-1 pathway. Incidentally, HBZ promotes also the cell cycle, activating the E2F1 pathway (reviewed in[157]).

HTLV-1 GAG AND UBIQUITINATION

Cells employ PTMs to organize trafficking activities and, in particular, ubiquitination plays a critical role in non-proteolytic functions such as DNA repair, exocytosis and endocytosis[158]. During the exocytosis, membrane scission requires the endosomal-sorting complex-required for transport (ESCRT) machinery and enveloped viruses exploit this system for virion budding[159]. Recently, evidences that arrestin-related trafficking proteins (ARTs, adaptors between WWP1, WWP2 and Itch ubiquitin ligases and ESCRT machinery) are recruited to the site of viral budding, suggest the involvement of ART-mediated ubiquitination in viral egress[160]. Early work by Derse and collaborators demonstrated that the HTLV-1 Gag matrix domain is involved in virion budding[161]. Gag matrix domain interacts through a late domain (PPxY motif: proline-proline-any amino acid-tyrosine) with WWP1 ubiquitin ligase (know also as NEDD4.1) and is ubiquitinated on the Lys-74 to function. Late domains are short peptide sequences encoded by enveloped viruses to promote the final separation of the nascent virus from the infected cell. Gag interacts with WWP1 at the plasma membrane where it is ubiquitinated and transferred to the late endosomes. There, it interacts with Tsg101, a component of the ESCRT machinery[162]. An ubiquitination-defective Gag mutant produces a substantial decrease in the virion release[161].

Furthermore, Gag binds also the tumor suppressor hDlg, mediating the cell-to-cell contact[163], one of the common mechanism of spreading for HTLV-1.

CONCLUDING REMARKS

The obligatory parasitic nature of viruses requires them to enter and hijack the cellular machinery with the contribution of viral encoded proteins, and to exploit the cellular resources for viral replication whilst blocking, inhibiting or balancing the host defenses.

HTLV-1 is capable of altering many cellular pathways and this phenomenon has been studied in immortalized cell lines and in *ex vivo* samples. Single or groups of viral proteins have been investigated *in vitro* or in cell culture under ectopic expression conditions. All the results are far from delineating the picture, but have highlighted a plethora of useful information about the regulation of cellular pathway through PTMs. HTLV-1 infection generates deregulation of many cellular proteins. Hsp-90 overexpression seems to favour Akt signaling and β-catenin-dependent cell proliferation. TCR signaling is hampered for the downregulation of CD3 and infected T cells seem to borrow components of the BCR pathway, making possible a conceptual parallelism between ATLL and BCLL. The downstream Ras-Raf-ERK pathway is turned on, suggesting the activation of MAP kinase and JNK as well. Jak/Stat pathway is constitutively activated in ATLL cells but is an unsuitable target for therapeutic treatments being redundant for proliferation.

Cell cycle is strictly controlled by PTMs and after infection is promoted by upregulation of Aurora kinases. It suffers major alterations for counteracting functions of upregulated cell proteins, Tax, HBZ and p30: while a G1 phase progression is promoted by Tax, Pin1 and HBZ mRNA, G2/M transition is blocked by Tax. Upregulated Kip1 and Waf1 inhibit G1/S/G2 transition but are takled by Akt activation. It seems that Tax acts, upstream and downstream, on Akt activation, promoting FoxO4 and p53 degradation and Bad inactivation, with the ultimate effect of altering cell cycle, response to DNA damage and apoptosis.

Tax function on gene transcription is hampered by p13, p30 and HBZ, but its activities on other cellular pathways, such as TCR, are far from being understood. Most likely, different Tax PTMs redirect Tax localization and function to specific compartments, where Tax is as essential as for transcription. For instance, the indirectly determined Tax phosphorylation on Ser-160 appears to stabilize the protein only during the mitosis, and the negative crosstalk between ubiquitination and SUMOylation on Lys-280 and Lys-284 seems to enable Tax shuttling between the cytoplasm and the nucleus. Centrosomes, ER and Golgi apparatus appears to be active site of recruitment for Tax and its cellular partners. Akt pathway, NF-κB pathway and DNA damage response require physical interaction between cell proteins and Tax. And also TCR signaling is probably affected directly by Tax for its putative SH3-binding motifs, as suggested by unpublished data (Pagano MA, personal communication) that show constitutive tyrosine phosphorylation in Tax-expressing cells. Like Tax-1, Tax2B undergoes to several PTMs, some of which have no discernible effects ascribed to date.

Other accessory proteins are essential to sustain the infection and the viral replication, and are subjected to modifications that could explain differences in localization and in function. Rex-1 and Rex-2 differ for phosphorylation residues and seems to work under phosphorylation. p12 induces NFAT activation while inhibiting the TCR pathway downstream Syk kinases, and its post-translational truncated form p8 localizes in the plasma membrane affecting TCR signaling and forming the virological synapse for viral spreading. p13 binds and constitutively activates SFKs, TCR effectors, suggesting an analogy with other leukemias involving overactivation of SFKs. Furthermore, a C-terminal truncated form of p13 and p30 in HTLV-1-infected cell promotes Vav hyperphosphorylation and a fatal disease in rabbit. At last, HBZ functions and Gag contribution in viral budding involve the recruitment of ubiquitin ligases.

Many HTLV-1 proteins affect and are modulated by cellular pathways implicated in phosphorylation, ubiquitination and other PTMs. One of the problems associated to the study of PTMs is that the proportion of modified residues in a specific protein could account for functional differences associated to the system (*in vivo, ex vivo, in culture* or *in vitro*), or associated to the described model (animal model, cell line). The application of the existing knowledge in several systems might solve partially the problem of recognizing relevant PTMs.

This data collection, not exhaustive of the clinical and molecular work done on HTLV-1, consents to appreciate the extensive interplay of the viral proteins with the host cellular machinery. The molecular mechanisms related to the virus replication are partially controlled by PTMs, that in turn are to some extent controlled by viral proteins. In ATLL, Akt pathway, downstream both Jak/Stat and TCR signaling, has revealed important in cell proliferation, survival and apoptosis abolition, together with Ras pathway. Both represent a possible target for pharmacological treatments. Jak/Stat instead has proven to be a pharmacological target only in specific leukemic settings. TCR signaling seems promising as a suitable pharmacological target. In ATLL, it is not responsive to external stimuli and is highly altered by the expression of a battery of enzymes proper of B cells, suggesting a parallelism between ATLL and BCLL. Hsp-90 could be involved in the overactivation of TCR signaling and, in particular, should be tested its putative involvement in SFK activation in ATLL. Furthermore, the identification of putative SH3-binding motifs in p12, p30 and Tax, and of an actual SH3-binding motif in p13 suggests the need to study the interplay between HTLV-1 proteins and SFKs, downstream TCR, as a prerequisite for the suitability of such kinases as pharmacological target.

Our knowledge is presently incomplete at least partly for the paucity of time-course studies on infected synchronized cells, which would extend the comprehension of the dynamic nature of PTMs. Further studies are needed that involve co-expression of viral proteins (or, alternatively, siRNA synchronous knockdown of more viral proteins within infected cells) to consider the balance between different and often opposite functions. More sophisticated lymphocyte models, with inducible expression of different HTLV-1 proteins, will provide new tools for the dissection of this fatal oncovirus. More insights and efforts are therefore needed to delineate novel mechanistic insights on viral exploitation of PTM pathways, which should provide a foundation for new treatment strategies to specifically target ATLL.

ACKNOWLEDGMENTS

The author thanks Noreen Sheehy for the critical reading of the paper, Michael Carr for the style refinement (colleagues at the Centre for Research in Infectious Diseases University College Dublin, Ireland) and Mario A Pagano (Department of Biological Chemistry, University of Padua, Italy) for the tenacious support and valuable comments. A special gratitude to Professor Umberto Bertazzoni and Professor William W Hall that substantially contributed to my professional advancement.

REFERENCES

1 **Kosako H**, Nagano K. Quantitative phosphoproteomics strategies for understanding protein kinase-mediated signal transduction pathways. *Expert Rev Proteomics* 2011; **8**: 81-94
2 **Hunter T**. The age of crosstalk: phosphorylation, ubiquitination, and beyond. *Mol Cell* 2007; **28**: 730-738
3 **Bhat KP**, Greer SF. Proteolytic and non-proteolytic roles of ubiquitin and the ubiquitin proteasome system in transcriptional regulation. *Biochim Biophys Acta* 2011; **1809**: 150-155
4 **Pozzebon M**, Segré CV, Chiocca S. Inhibition of the SUMO pathway by Gam1. *Methods Mol Biol* 2009; **497**: 285-301
5 **Zhao Y**, Wang Y, Zhu WG. Applications of post-translational modifications of FoxO family proteins in biological functions. *J Mol Cell Biol* 2011; **3**: 276-282
6 **Shen F**, Kirmani KZ, Xiao Z, Thirlby BH, Hickey RJ, Malkas LH. Nuclear protein isoforms: implications for cancer diagnosis and therapy. *J Cell Biochem* 2011; **112**: 756-760

7 **Van Duyne R**, Kehn-Hall K, Carpio L, Kashanchi F. Cell-type-specific proteome and interactome: using HIV-1 Tat as a test case. *Expert Rev Proteomics* 2009; **6**: 515-526
8 **Hu X**, Moscinski LC. Cdc2: a monopotent or pluripotent CDK? *Cell Prolif* 2011; **44**: 205-211
9 **Svoboda J**, Hejnar J, Geryk J, Elleder D, Vernerová Z. Retroviruses in foreign species and the problem of provirus silencing. *Gene* 2000; **261**: 181-188
10 **Takeda S**, Maeda M, Morikawa S, Taniguchi Y, Yasunaga J, Nosaka K, Tanaka Y, Matsuoka M. Genetic and epigenetic inactivation of tax gene in adult T-cell leukemia cells. *Int J Cancer* 2004; **109**: 559-567
11 **Kamoi K**, Yamamoto K, Misawa A, Miyake A, Ishida T, Tanaka Y, Mochizuki M, Watanabe T. SUV39H1 interacts with HTLV-1 Tax and abrogates Tax transactivation of HTLV-1 LTR. *Retrovirology* 2006; **3**: 5
12 **Lemasson I**, Polakowski NJ, Laybourn PJ, Nyborg JK. Transcription factor binding and histone modifications on the integrated proviral promoter in human T-cell leukemia virus-I-infected T-cells. *J Biol Chem* 2002; **277**: 49459-49465
13 **Lu H**, Pise-Masison CA, Fletcher TM, Schiltz RL, Nagaich AK, Radonovich M, Hager G, Cole PA, Brady JN. Acetylation of nucleosomal histones by p300 facilitates transcription from tax-responsive human T-cell leukemia virus type 1 chromatin template. *Mol Cell Biol* 2002; **22**: 4450-4462
14 **Georges SA**, Kraus WL, Luger K, Nyborg JK, Laybourn PJ. p300-mediated tax transactivation from recombinant chromatin: histone tail deletion mimics coactivator function. *Mol Cell Biol* 2002; **22**: 127-137
15 **Chen J**, Zhang M, Ju W, Waldmann TA. Effective treatment of a murine model of adult T-cell leukemia using depsipeptide and its combination with unmodified daclizumab directed toward CD25. *Blood* 2009; **113**: 1287-1293
16 **Easley R**, Carpio L, Guendel I, Klase Z, Choi S, Kehn-Hall K, Brady JN, Kashanchi F. Human T-lymphotropic virus type 1 transcription and chromatin-remodeling complexes. *J Virol* 2010; **84**: 4755-4768
17 Abstracts of the 15th International Conference on Human Retroviruses: HTLV and Related Viruses. Leuven and Gembloux, Belgium. June 5-8, 2011. *Retrovirology* 2011; **8** Suppl 1: A2-254
18 **Sadoul K**, Wang J, Diagouraga B, Khochbin S. The tale of protein lysine acetylation in the cytoplasm. *J Biomed Biotechnol* 2011; **2011**: 970382
19 **Shembade N**, Harhaj EW. Role of post-translational modifications of HTLV-1 Tax in NF-κB activation. *World J Biol Chem* 2010; **1**: 13-20
20 **Journo C**, Douceron E, Mahieux R. HTLV gene regulation: because size matters, transcription is not enough. *Future Microbiol* 2009; **4**: 425-440
21 **Viswanathan K**, Früh K, DeFilippis V. Viral hijacking of the host ubiquitin system to evade interferon responses. *Curr Opin Microbiol* 2010; **13**: 517-523
22 **Wang S**, Fischer PM. Cyclin-dependent kinase 9: a key transcriptional regulator and potential drug target in oncology, virology and cardiology. *Trends Pharmacol Sci* 2008; **29**: 302-313
23 **Mori N**. Cell signaling modifiers for molecular targeted therapy in ATLL. *Front Biosci* 2009; **14**: 1479-1489
24 **Grassmann R**, Berchtold S, Radant I, Alt M, Fleckenstein B, Sodroski JG, Haseltine WA, Ramstedt U. Role of human T-cell leukemia virus type 1 X region proteins in immortalization of primary human lymphocytes in culture. *J Virol* 1992; **66**: 4570-4575
25 **Nicot C**, Harrod RL, Ciminale V, Franchini G. Human T-cell leukemia/lymphoma virus type 1 nonstructural genes and their functions. *Oncogene* 2005; **24**: 6026-6034
26 **Grassmann R**, Aboud M, Jeang KT. Molecular mechanisms of cellular transformation by HTLV-1 Tax. *Oncogene* 2005; **24**: 5976-5985
27 **Edwards D**, Fenizia C, Gold H, de Castro-Amarante MF, Buchmann C, Pise-Masison CA, Franchini G. Orf-I and orf-II-encoded proteins in HTLV-1 infection and persistence. *Viruses* 2011; **3**: 861-885
28 **Arnold J**, Zimmerman B, Li M, Lairmore MD, Green PL. Human T-cell leukemia virus type-1 antisense-encoded gene, Hbz, promotes T-lymphocyte proliferation. *Blood* 2008; **112**: 3788-3797
29 **Barbeau B**, Mesnard JM. Making sense out of antisense transcription in human T-cell lymphotropic viruses (HTLVs). *Viruses* 2011; **3**: 456-468
30 **Maldonado H**, Ortiz-Riaño E, Krause B, Barriga A, Medina F, Pando ME, Alberti C, Kettlun AM, Collados L, García L, Cartier L, Valenzuela MA. Microtubule proteins and their post-translational forms in the cerebrospinal fluid of patients with paraparesis associated with HTLV-I infection and in SH-SY5Y cells: an in vitro model of HTLV-I-induced disease. *Biol Res* 2008; **41**: 239-259
31 **Maldonado H**, Ramírez E, Utreras E, Pando ME, Kettlun AM, Chiong M, Kulkarni AB, Collados L, Puente J, Cartier L, Valenzuela MA. Inhibition of cyclin-dependent kinase 5 but not of glycogen synthase kinase 3-β prevents neurite retraction and tau hyperphosphorylation caused by secretable products of human T-cell leukemia virus type I-infected lymphocytes. *J Neurosci Res* 2011; **89**: 1489-1498
32 **Kawakami H**, Tomita M, Okudaira T, Ishikawa C, Matsuda T, Tanaka Y, Nakazato T, Taira N, Ohshiro K, Mori N. Inhibition of heat shock protein-90 modulates multiple functions required for survival of human T-cell leukemia virus type I-infected T-cell lines and adult T-cell leukemia cells. *Int J Cancer* 2007; **120**: 1811-1820
33 **Kurashina R**, Ohyashiki JH, Kobayashi C, Hamamura R, Zhang Y, Hirano T, Ohyashiki K. Anti-proliferative activity of heat shock protein (Hsp) 90 inhibitors via beta-catenin/TCF7L2 pathway in adult T cell leukemia cells. *Cancer Lett* 2009; **284**: 62-70
34 **Liu C**, Kato Y, Zhang Z, Do VM, Yankner BA, He X. beta-Trcp couples beta-catenin phosphorylation-degradation and regulates Xenopus axis formation. *Proc Natl Acad Sci USA* 1999; **96**: 6273-6278
35 **Tomita M**, Kikuchi A, Akiyama T, Tanaka Y, Mori N. Human T-cell leukemia virus type 1 tax dysregulates beta-catenin signaling. *J Virol* 2006; **80**: 10497-10505
36 **Trentin L**, Frasson M, Donella-Deana A, Frezzato F, Pagano MA, Tibaldi E, Gattazzo C, Zambello R, Semenzato G, Brunati AM. Geldanamycin-induced Lyn dissociation from aberrant Hsp90-stabilized cytosolic complex is an early event in apoptotic mechanisms in B-chronic lymphocytic leukemia. *Blood* 2008; **112**: 4665-4674
37 **Shuh M**, Morse BA, Heidecker G, Derse D. Association of SRC-related kinase Lyn with the interleukin-2 receptor and its role in maintaining constitutive phosphorylation of JAK/STAT in human T-cell leukemia virus type 1-transformed T cells. *J Virol* 2011; **85**: 4623-4627
38 **Jerome KR**. Viral modulation of T-cell receptor signaling. *J Virol* 2008; **82**: 4194-4204
39 **Jeong SJ**, Dasgupta A, Jung KJ, Um JH, Burke A, Park HU, Brady JN. PI3K/AKT inhibition induces caspase-dependent apoptosis in HTLV-1-transformed cells. *Virology* 2008; **370**: 264-272
40 **Lemasson I**, Robert-Hebmann V, Hamaia S, Duc Dodon M, Gazzolo L, Devaux C. Transrepression of lck gene expression by human T-cell leukemia virus type 1-encoded p40tax. *J Virol* 1997; **71**: 1975-1983
41 **Weil R**, Levraud JP, Dodon MD, Bessia C, Hazan U, Kourilsky P, Israël A. Altered expression of tyrosine kinases of the Src and Syk families in human T-cell leukemia virus type 1-infected T-cell lines. *J Virol* 1999; **73**: 3709-3717
42 **Lin J**, Weiss A. T cell receptor signalling. *J Cell Sci* 2001; **114**: 243-244

43 **Dent P**, Yacoub A, Fisher PB, Hagan MP, Grant S. MAPK pathways in radiation responses. *Oncogene* 2003; **22**: 5885-5896
44 **Nonaka M**, Uota S, Saitoh Y, Takahashi M, Sugimoto H, Amet T, Arai A, Miura O, Yamamoto N, Yamaoka S. Role for protein geranylgeranylation in adult T-cell leukemia cell survival. *Exp Cell Res* 2009; **315**: 141-150
45 **Morgan MA**, Sebil T, Aydilek E, Peest D, Ganser A, Reuter CW. Combining prenylation inhibitors causes synergistic cytotoxicity, apoptosis and disruption of RAS-to-MAP kinase signalling in multiple myeloma cells. *Br J Haematol* 2005; **130**: 912-925
46 **Song C**, Wang W, Li M, Liu Y, Zheng D. Tax1 enhances cancer cell proliferation via Ras-Raf-MEK-ERK signaling pathway. *IUBMB Life* 2009; **61**: 685-692
47 **Vajente N**, Trevisan R, Saggioro D. HTLV-1 Tax protein cooperates with Ras in protecting cells from apoptosis. *Apoptosis* 2009; **14**: 153-163
48 **Huang YZ**, Zang M, Xiong WC, Luo Z, Mei L. Erbin suppresses the MAP kinase pathway. *J Biol Chem* 2003; **278**: 1108-1114
49 **Takemoto S**, Mulloy JC, Cereseto A, Migone TS, Patel BK, Matsuoka M, Yamaguchi K, Takatsuki K, Kamihira S, White JD, Leonard WJ, Waldmann T, Franchini G. Proliferation of adult T cell leukemia/lymphoma cells is associated with the constitutive activation of JAK/STAT proteins. *Proc Natl Acad Sci USA* 1997; **94**: 13897-13902
50 **Imada K**, Leonard WJ. The Jak-STAT pathway. *Mol Immunol* 2000; **37**: 1-11
51 **Migone TS**, Lin JX, Cereseto A, Mulloy JC, O'Shea JJ, Franchini G, Leonard WJ. Constitutively activated Jak-STAT pathway in T cells transformed with HTLV-I. *Science* 1995; **269**: 79-81
52 **Mulloy JC**, Migone TS, Ross TM, Ton N, Green PL, Leonard WJ, Franchini G. Human and simian T-cell leukemia viruses type 2 (HTLV-2 and STLV-2(pan-p)) transform T cells independently of Jak/STAT activation. *J Virol* 1998; **72**: 4408-4412
53 **Taylor JM**, Brown M, Nejmeddine M, Kim KJ, Ratner L, Lairmore M, Nicot C. Novel role for interleukin-2 receptor-Jak signaling in retrovirus transmission. *J Virol* 2009; **83**: 11467-11476
54 **Samaan A**, Mahana W. Constitutive and induced activation of JAK/Stat pathway in leukemogenic and asymptomatic human T-cell lymphoptropic virus type 1 (HTLV-1) transformed rabbit cell lines. *Immunol Lett* 2007; **109**: 113-119
55 **Feng X**, Ratner L. Human T-cell leukemia virus type 1 blunts signaling by interferon alpha. *Virology* 2008; **374**: 210-216
56 **Kirken RA**, Erwin RA, Wang L, Wang Y, Rui H, Farrar WL. Functional uncoupling of the Janus kinase 3-Stat5 pathway in malignant growth of human T cell leukemia virus type 1-transformed human T cells. *J Immunol* 2000; **165**: 5097-5104
57 **Ju W**, Zhang M, Jiang JK, Thomas CJ, Oh U, Bryant BR, Chen J, Sato N, Tagaya Y, Morris JC, Janik JE, Jacobson S, Waldmann TA. CP-690,550, a therapeutic agent, inhibits cytokine-mediated Jak3 activation and proliferation of T cells from patients with ATL and HAM/TSP. *Blood* 2011; **117**: 1938-1946
58 **Olière S**, Hernandez E, Lézin A, Arguello M, Douville R, Nguyen TL, Olindo S, Panelatti G, Kazanji M, Wilkinson P, Sékaly RP, Césaire R, Hiscott J. HTLV-1 evades type I interferon antiviral signaling by inducing the suppressor of cytokine signaling 1 (SOCS1). *PLoS Pathog* 2010; **6**: e1001177
59 **Barry M**, Früh K. Viral modulators of cullin RING ubiquitin ligases: culling the host defense. *Sci STKE* 2006; **2006**: pe21
60 **Liu M**, Yang L, Zhang L, Liu B, Merling R, Xia Z, Giam CZ. Human T-cell leukemia virus type 1 infection leads to arrest in the G1 phase of the cell cycle. *J Virol* 2008; **82**: 8442-8455
61 **de La Fuente C**, Santiago F, Chong SY, Deng L, Mayhood T, Fu P, Stein D, Denny T, Coffman F, Azimi N, Mahieux R, Kashanchi F. Overexpression of p21(waf1) in human T-cell lymphotropic virus type 1-infected cells and its association with cyclin A/cdk2. *J Virol* 2000; **74**: 7270-7283
62 **Wu CL**, Zukerberg LR, Ngwu C, Harlow E, Lees JA. In vivo association of E2F and DP family proteins. *Mol Cell Biol* 1995; **15**: 2536-2546
63 **Cereseto A**, Washington Parks R, Rivadeneira E, Franchini G. Limiting amounts of p27Kip1 correlates with constitutive activation of cyclin E-CDK2 complex in HTLV-I-transformed T-cells. *Oncogene* 1999; **18**: 2441-2450
64 **Bolanos-Garcia VM**. Aurora kinases. *Int J Biochem Cell Biol* 2005; **37**: 1572-1577
65 **Tomita M**, Toyota M, Ishikawa C, Nakazato T, Okudaira T, Matsuda T, Uchihara JN, Taira N, Ohshiro K, Senba M, Tanaka Y, Ohshima K, Saya H, Tokino T, Mori N. Overexpression of Aurora A by loss of CHFR gene expression increases the growth and survival of HTLV-1-infected T cells through enhanced NF-kappaB activity. *Int J Cancer* 2009; **124**: 2607-2615
66 **Tomita M**, Tanaka Y, Mori N. Aurora kinase inhibitor AZD1152 negatively affects the growth and survival of HTLV-1-infected T lymphocytes in vitro. *Int J Cancer* 2010; **127**: 1584-1594
67 **Kfoury Y**, Setterblad N, El-Sabban M, Zamborlini A, Dassouki Z, El Hajj H, Hermine O, Pique C, de Thé H, Saïb A, Bazarbachi A. Tax ubiquitylation and SUMOylation control the dynamic shuttling of Tax and NEMO between Ubc9 nuclear bodies and the centrosome. *Blood* 2011; **117**: 190-199
68 **Bex F**, Murphy K, Wattiez R, Burny A, Gaynor RB. Phosphorylation of the human T-cell leukemia virus type 1 transactivator tax on adjacent serine residues is critical for tax activation. *J Virol* 1999; **73**: 738-745
69 **Durkin SS**, Ward MD, Fryrear KA, Semmes OJ. Site-specific phosphorylation differentiates active from inactive forms of the human T-cell leukemia virus type 1 Tax oncoprotein. *J Biol Chem* 2006; **281**: 31705-31712
70 **Bidoia C**, Mazzorana M, Pagano MA, Arrigoni G, Meggio F, Pinna LA, Bertazzoni U. The pleiotropic protein kinase CK2 phosphorylates HTLV-1 Tax protein in vitro, targeting its PDZ-binding motif. *Virus Genes* 2010; **41**: 149-157
71 **Lodewick J**, Lamsoul I, Polania A, Lebrun S, Burny A, Ratner L, Bex F. Acetylation of the human T-cell leukemia virus type 1 Tax oncoprotein by p300 promotes activation of the NF-kappaB pathway. *Virology* 2009; **386**: 68-78
72 **Chiari E**, Lamsoul I, Lodewick J, Chopin C, Bex F, Pique C. Stable ubiquitination of human T-cell leukemia virus type 1 tax is required for proteasome binding. *J Virol* 2004; **78**: 11823-11832
73 **Sun SC**, Harhaj EW, Xiao G, Good L. Activation of I-kappaB kinase by the HTLV type 1 Tax protein: mechanistic insights into the adaptor function of IKKgamma. *AIDS Res Hum Retroviruses* 2000; **16**: 1591-1596
74 **Nasr R**, Chiari E, El-Sabban M, Mahieux R, Kfoury Y, Abdulhay M, Yazbeck V, Hermine O, de Thé H, Pique C, Bazarbachi A. Tax ubiquitylation and sumoylation control critical cytoplasmic and nuclear steps of NF-kappaB activation. *Blood* 2006; **107**: 4021-4029
75 **Lamsoul I**, Lodewick J, Lebrun S, Brasseur R, Burny A, Gaynor RB, Bex F. Exclusive ubiquitination and sumoylation on overlapping lysine residues mediate NF-kappaB activation by the human T-cell leukemia virus tax oncoprotein. *Mol Cell Biol* 2005; **25**: 10391-10406
76 **Peloponese JM**, Iha H, Yedavalli VR, Miyazato A, Li Y, Haller K, Benkirane M, Jeang KT. Ubiquitination of human T-cell leukemia virus type 1 tax modulates its activity. *J Virol* 2004; **78**: 11686-11695
77 **Harhaj NS**, Sun SC, Harhaj EW. Activation of NF-kappa B by the human T cell leukemia virus type I Tax oncoprotein is associated with ubiquitin-dependent relocalization of I kappa B kinase. *J Biol Chem* 2007; **282**: 4185-4192
78 **Harrod R**, Tang Y, Nicot C, Lu HS, Vassilev A, Nakatani Y, Giam CZ. An exposed KID-like domain in human T-cell lym-

photropic virus type 1 Tax is responsible for the recruitment of coactivators CBP/p300. *Mol Cell Biol* 1998; **18**: 5052-5061
79 **Kfoury Y**, Nasr R, Favre-Bonvin A, El-Sabban M, Renault N, Giron ML, Setterblad N, Hajj HE, Chiari E, Mikati AG, Hermine O, Saib A, de Thé H, Pique C, Bazarbachi A. Ubiquitylated Tax targets and binds the IKK signalosome at the centrosome. *Oncogene* 2008; **27**: 1665-1676
80 **Yu Q**, Minoda Y, Yoshida R, Yoshida H, Iha H, Kobayashi T, Yoshimura A, Takaesu G. HTLV-1 Tax-mediated TAK1 activation involves TAB2 adapter protein. *Biochem Biophys Res Commun* 2008; **365**: 189-194
81 **Inoue J**, Ishida T, Tsukamoto N, Kobayashi N, Naito A, Azuma S, Yamamoto T. Tumor necrosis factor receptor-associated factor (TRAF) family: adapter proteins that mediate cytokine signaling. *Exp Cell Res* 2000; **254**: 14-24
82 **Yan P**, Fu J, Qu Z, Li S, Tanaka T, Grusby MJ, Xiao G. PDLIM2 suppresses human T-cell leukemia virus type I Tax-mediated tumorigenesis by targeting Tax into the nuclear matrix for proteasomal degradation. *Blood* 2009; **113**: 4370-4380
83 **Shembade N**, Harhaj NS, Yamamoto M, Akira S, Harhaj EW. The human T-cell leukemia virus type 1 Tax oncoprotein requires the ubiquitin-conjugating enzyme Ubc13 for NF-kappaB activation. *J Virol* 2007; **81**: 13735-13742
84 **Journo C**, Filipe J, About F, Chevalier SA, Afonso PV, Brady JN, Flynn D, Tangy F, Israël A, Vidalain PO, Mahieux R, Weil R. NRP/Optineurin Cooperates with TAX1BP1 to potentiate the activation of NF-kappaB by human T-lymphotropic virus type 1 tax protein. *PLoS Pathog* 2009; **5**: e1000521
85 **Yasunaga J**, Lin FC, Lu X, Jeang KT. Ubiquitin-specific peptidase 20 targets TRAF6 and human T cell leukemia virus type 1 tax to negatively regulate NF-kappaB signaling. *J Virol* 2011; **85**: 6212-6219
86 **Gatza ML**, Dayaram T, Marriott SJ. Ubiquitination of HTLV-I Tax in response to DNA damage regulates nuclear complex formation and nuclear export. *Retrovirology* 2007; **4**: 95
87 **Jeong SJ**, Ryo A, Yamamoto N. The prolyl isomerase Pin1 stabilizes the human T-cell leukemia virus type 1 (HTLV-1) Tax oncoprotein and promotes malignant transformation. *Biochem Biophys Res Commun* 2009; **381**: 294-299
88 **Peloponese JM**, Yasunaga J, Kinjo T, Watashi K, Jeang KT. Peptidylproline cis-trans-isomerase Pin1 interacts with human T-cell leukemia virus type 1 tax and modulates its activation of NF-kappaB. *J Virol* 2009; **83**: 3238-3248
89 **Lu KP**, Zhou XZ. The prolyl isomerase PIN1: a pivotal new twist in phosphorylation signalling and disease. *Nat Rev Mol Cell Biol* 2007; **8**: 904-916
90 **Suizu F**, Ryo A, Wulf G, Lim J, Lu KP. Pin1 regulates centrosome duplication, and its overexpression induces centrosome amplification, chromosome instability, and oncogenesis. *Mol Cell Biol* 2006; **26**: 1463-1479
91 **Kim YM**, Geiger TR, Egan DI, Sharma N, Nyborg JK. The HTLV-1 tax protein cooperates with phosphorylated CREB, TORC2 and p300 to activate CRE-dependent cyclin D1 transcription. *Oncogene* 2010; **29**: 2142-2152
92 **Liu B**, Hong S, Tang Z, Yu H, Giam CZ. HTLV-I Tax directly binds the Cdc20-associated anaphase-promoting complex and activates it ahead of schedule. *Proc Natl Acad Sci USA* 2005; **102**: 63-68
93 **Nyunoya H**, Akagi T, Ogura T, Maeda S, Shimotohno K. Evidence for phosphorylation of trans-activator p40x of human T-cell leukemia virus type I produced in insect cells with a baculovirus expression vector. *Virology* 1988; **167**: 538-544
94 **Fontes JD**, Strawhecker JM, Bills ND, Lewis RE, Hinrichs SH. Phorbol esters modulate the phosphorylation of human T-cell leukemia virus type I Tax. *J Virol* 1993; **67**: 4436-4441
95 **Smith MR**, Greene WC. Identification of HTLV-I tax trans-activator mutants exhibiting novel transcriptional phenotypes. *Genes Dev* 1990; **4**: 1875-1885
96 **Semmes OJ**, Jeang KT. Mutational analysis of human T-cell leukemia virus type I Tax: regions necessary for function determined with 47 mutant proteins. *J Virol* 1992; **66**: 7183-7192
97 **Krause Boehm A**, Stawhecker JA, Semmes OJ, Jankowski PE, Lewis R, Hinrichs SH. Analysis of potential phosphorylation sites in human T cell leukemia virus type 1 Tax. *J Biomed Sci* 1999; **6**: 206-212
98 **Higuchi M**, Fujii M. Distinct functions of HTLV-1 Tax1 from HTLV-2 Tax2 contribute key roles to viral pathogenesis. *Retrovirology* 2009; **6**: 117
99 **Bertazzoni U**, Turci M, Avesani F, Di Gennaro G, Bidoia C, Romanelli MG. Intracellular localization and cellular factors interaction of HTLV-1 and HTLV-2 Tax proteins: similarities and functional differences. *Viruses* 2011; **3**: 541-560
100 **Turci M**, Lodewick J, Righi P, Polania A, Romanelli MG, Bex F, Bertazzoni U. HTLV-2B Tax oncoprotein is modified by ubiquitination and sumoylation and displays intracellular localization similar to its homologue HTLV-1 Tax. *Virology* 2009; **386**: 6-11
101 **Xiao G**, Cvijic ME, Fong A, Harhaj EW, Uhlik MT, Waterfield M, Sun SC. Retroviral oncoprotein Tax induces processing of NF-kappaB2/p100 in T cells: evidence for the involvement of IKKalpha. *EMBO J* 2001; **20**: 6805-6815
102 **Qu Z**, Qing G, Rabson A, Xiao G. Tax deregulation of NF-kappaB2 p100 processing involves both beta-TrCP-dependent and -independent mechanisms. *J Biol Chem* 2004; **279**: 44563-44572
103 **Spencer E**, Jiang J, Chen ZJ. Signal-induced ubiquitination of IkappaBalpha by the F-box protein Slimb/beta-TrCP. *Genes Dev* 1999; **13**: 284-294
104 **Yin MJ**, Christerson LB, Yamamoto Y, Kwak YT, Xu S, Mercurio F, Barbosa M, Cobb MH, Gaynor RB. HTLV-I Tax protein binds to MEKK1 to stimulate IkappaB kinase activity and NF-kappaB activation. *Cell* 1998; **93**: 875-884
105 **Wada K**, Niida M, Tanaka M, Kamitani T. Ro52-mediated monoubiquitination of IKK{beta} down-regulates NF-{kappa}B signalling. *J Biochem* 2009; **146**: 821-832
106 **Niida M**, Tanaka M, Kamitani T. Downregulation of active IKK beta by Ro52-mediated autophagy. *Mol Immunol* 2010; **47**: 2378-2387
107 **Petropoulos L**, Hiscott J. Association between HTLV-1 Tax and I kappa B alpha is dependent on the I kappa B alpha phosphorylation state. *Virology* 1998; **252**: 189-199
108 **Wu X**, Sun SC. Retroviral oncoprotein Tax deregulates NF-kappaB by activating Tak1 and mediating the physical association of Tak1-IKK. *EMBO Rep* 2007; **8**: 510-515
109 **Suzuki S**, Singhirunnusorn P, Mori A, Yamaoka S, Kitajima I, Saiki I, Sakurai H. Constitutive activation of TAK1 by HTLV-1 tax-dependent overexpression of TAB2 induces activation of JNK-ATF2 but not IKK-NF-kappaB. *J Biol Chem* 2007; **282**: 25177-25181
110 **Avesani F**, Romanelli MG, Turci M, Di Gennaro G, Sampaio C, Bidoia C, Bertazzoni U, Bex F. Association of HTLV Tax proteins with TAK1-binding protein 2 and RelA in calreticulin-containing cytoplasmic structures participates in Tax-mediated NF-κB activation. *Virology* 2010; **408**: 39-48
111 **Washiyama M**, Nishigaki K, Ahmed N, Kinpara S, Ishii Y, Kanzawa N, Masuda T, Kannagi M. IL-2 withdrawal induces HTLV-1 expression through p38 activation in ATL cell lines. *FEBS Lett* 2007; **581**: 5207-5212
112 **Zhao T**, Yasunaga J, Satou Y, Nakao M, Takahashi M, Fujii M, Matsuoka M. Human T-cell leukemia virus type 1 bZIP factor selectively suppresses the classical pathway of NF-kappaB. *Blood* 2009; **113**: 2755-2764
113 **Oteiza A**, Mechti N. The human T-cell leukemia virus type 1 oncoprotein tax controls forkhead box O4 activity through degradation by the proteasome. *J Virol* 2011; **85**: 6480-6491
114 **Pise-Masison CA**, Radonovich M, Sakaguchi K, Appella E, Brady JN. Phosphorylation of p53: a novel pathway for p53 inactivation in human T-cell lymphotropic virus type

1-transformed cells. *J Virol* 1998; **72**: 6348-6355
115 **Datta A**, Nicot C. Telomere attrition induces a DNA double-strand break damage signal that reactivates p53 transcription in HTLV-I leukemic cells. *Oncogene* 2008; **27**: 1135-1141
116 **Pise-Masison CA**, Mahieux R, Jiang H, Ashcroft M, Radonovich M, Duvall J, Guillerm C, Brady JN. Inactivation of p53 by human T-cell lymphotropic virus type 1 Tax requires activation of the NF-kappaB pathway and is dependent on p53 phosphorylation. *Mol Cell Biol* 2000; **20**: 3377-3386
117 **Ariumi Y**, Kaida A, Lin JY, Hirota M, Masui O, Yamaoka S, Taya Y, Shimotohno K. HTLV-1 tax oncoprotein represses the p53-mediated trans-activation function through coactivator CBP sequestration. *Oncogene* 2000; **19**: 1491-1499
118 **Mulloy JC**, Kislyakova T, Cereseto A, Casareto L, LoMonico A, Fullen J, Lorenzi MV, Cara A, Nicot C, Giam C, Franchini G. Human T-cell lymphotropic/leukemia virus type 1 Tax abrogates p53-induced cell cycle arrest and apoptosis through its CREB/ATF functional domain. *J Virol* 1998; **72**: 8852-8860
119 **Mahieux R**, Pise-Masison CA, Lambert PF, Nicot C, De Marchis L, Gessain A, Green P, Hall W, Brady JN. Differences in the ability of human T-cell lymphotropic virus type 1 (HTLV-1) and HTLV-2 tax to inhibit p53 function. *J Virol* 2000; **74**: 6866-6874
120 **Bellon M**, Baydoun HH, Yao Y, Nicot C. HTLV-I Tax-dependent and -independent events associated with immortalization of human primary T lymphocytes. *Blood* 2010; **115**: 2441-2448
121 **Akl H**, Badran BM, Zein NE, Bex F, Sotiriou C, Willard-Gallo KE, Burny A, Martiat P. HTLV-I infection of WE17/10 CD4+ cell line leads to progressive alteration of Ca2+ influx that eventually results in loss of CD7 expression and activation of an antiapoptotic pathway involving AKT and BAD which paves the way for malignant transformation. *Leukemia* 2007; **21**: 788-796
122 **Nyborg JK**, Egan D, Sharma N. The HTLV-1 Tax protein: revealing mechanisms of transcriptional activation through histone acetylation and nucleosome disassembly. *Biochim Biophys Acta* 2010; **1799**: 266-274
123 **Sharma N**, Nyborg JK. The coactivators CBP/p300 and the histone chaperone NAP1 promote transcription-independent nucleosome eviction at the HTLV-1 promoter. *Proc Natl Acad Sci USA* 2008; **105**: 7959-7963
124 **Cheng J**, Kydd AR, Nakase K, Noonan KM, Murakami A, Tao H, Dwyer M, Xu C, Zhu Q, Marasco WA. Negative regulation of the SH2-homology containing protein-tyrosine phosphatase-1 (SHP-1) P2 promoter by the HTLV-1 Tax oncoprotein. *Blood* 2007; **110**: 2110-2120
125 **Migone TS**, Cacalano NA, Taylor N, Yi T, Waldmann TA, Johnston JA. Recruitment of SH2-containing protein tyrosine phosphatase SHP-1 to the interleukin 2 receptor; loss of SHP-1 expression in human T-lymphotropic virus type I-transformed T cells. *Proc Natl Acad Sci USA* 1998; **95**: 3845-3850
126 **Zhang Q**, Lee B, Korecka M, Li G, Weyland C, Eck S, Gessain A, Arima N, Lessin SR, Shaw LM, Luger S, Kamoun M, Wasik MA. Differences in phosphorylation of the IL-2R associated JAK/STAT proteins between HTLV-I(+), IL-2-independent and IL-2-dependent cell lines and uncultured leukemic cells from patients with adult T-cell lymphoma/leukemia. *Leuk Res* 1999; **23**: 373-384
127 **Villanueva R**, Iglesias AH, Camelo S, Sanin LC, Gray SG, Dangond F. Histone deacetylase 3 represses HTLV-1 tax transcription. *Oncol Rep* 2006; **16**: 581-585
128 **Guyot DJ**, Newbound GC, Lairmore MD. Signaling via the CD2 receptor enhances HTLV-1 replication in T lymphocytes. *Virology* 1997; **234**: 123-129
129 **Guyot DJ**, Newbound GC, Lairmore MD. CD2 signalling induces phosphorylation of CREB in primary lymphocytes. *Immunology* 1998; **95**: 544-552
130 **Kim YM**, Ramírez JA, Mick JE, Giebler HA, Yan JP, Nyborg JK. Molecular characterization of the Tax-containing HTLV-1 enhancer complex reveals a prominent role for CREB phosphorylation in Tax transactivation. *J Biol Chem* 2007; **282**: 18750-18757
131 **Gachon F**, Peleraux A, Thebault S, Dick J, Lemasson I, Devaux C, Mesnard JM. CREB-2, a cellular CRE-dependent transcription repressor, functions in association with Tax as an activator of the human T-cell leukemia virus type 1 promoter. *J Virol* 1998; **72**: 8332-8337
132 **Durkin SS**, Guo X, Fryrear KA, Mihaylova VT, Gupta SK, Belgnaoui SM, Haoudi A, Kupfer GM, Semmes OJ. HTLV-1 Tax oncoprotein subverts the cellular DNA damage response via binding to DNA-dependent protein kinase. *J Biol Chem* 2008; **283**: 36311-36320
133 **Younis I**, Green PL. The human T-cell leukemia virus Rex protein. *Front Biosci* 2005; **10**: 431-445
134 **Adachi Y**, Copeland TD, Takahashi C, Nosaka T, Ahmed A, Oroszlan S, Hatanaka M. Phosphorylation of the Rex protein of human T-cell leukemia virus type I. *J Biol Chem* 1992; **267**: 21977-21981
135 **Kesic M**, Doueiri R, Ward M, Semmes OJ, Green PL. Phosphorylation regulates human T-cell leukemia virus type 1 Rex function. *Retrovirology* 2009; **6**: 105
136 **Kesic M**, Ward M, Semmes OJ, Green PL. Site-specific phosphorylation regulates human T-cell leukemia virus type 2 Rex function in vivo. *J Virol* 2009; **83**: 8859-8868
137 **Xie L**, Kesic M, Yamamoto B, Li M, Younis I, Lairmore MD, Green PL. Human T-cell leukemia virus type 2 Rex carboxy terminus is an inhibitory/stability domain that regulates Rex functional activity and viral replication. *J Virol* 2009; **83**: 5232-5243
138 **Nair AM**, Michael B, Datta A, Fernandez S, Lairmore MD. Calcium-dependent enhancement of transcription of p300 by human T-lymphotropic type 1 p12I. *Virology* 2006; **353**: 247-257
139 **Michael B**, Nair AM, Datta A, Hiraragi H, Ratner L, Lairmore MD. Histone acetyltransferase (HAT) activity of p300 modulates human T lymphotropic virus type 1 p30II-mediated repression of LTR transcriptional activity. *Virology* 2006; **354**: 225-239
140 **Collins ND**, D'Souza C, Albrecht B, Robek MD, Ratner L, Ding W, Green PL, Lairmore MD. Proliferation response to interleukin-2 and Jak/Stat activation of T cells immortalized by human T-cell lymphotropic virus type 1 is independent of open reading frame I expression. *J Virol* 1999; **73**: 9642-9649
141 **Nicot C**, Mulloy JC, Ferrari MG, Johnson JM, Fu K, Fukumoto R, Trovato R, Fullen J, Leonard WJ, Franchini G. HTLV-1 p12(I) protein enhances STAT5 activation and decreases the interleukin-2 requirement for proliferation of primary human peripheral blood mononuclear cells. *Blood* 2001; **98**: 823-829
142 **Albrecht B**, D'Souza CD, Ding W, Tridandapani S, Coggeshall KM, Lairmore MD. Activation of nuclear factor of activated T cells by human T-lymphotropic virus type 1 accessory protein p12(I). *J Virol* 2002; **76**: 3493-3501
143 **Fukumoto R**, Dundr M, Nicot C, Adams A, Valeri VW, Samelson LE, Franchini G. Inhibition of T-cell receptor signal transduction and viral expression by the linker for activation of T cells-interacting p12(I) protein of human T-cell leukemia/lymphoma virus type 1. *J Virol* 2007; **81**: 9088-9099
144 **Van Prooyen N**, Gold H, Andresen V, Schwartz O, Jones K, Ruscetti F, Lockett S, Gudla P, Venzon D, Franchini G. Human T-cell leukemia virus type 1 p8 protein increases cellular conduits and virus transmission. *Proc Natl Acad Sci USA* 2010; **107**: 20738-20743
145 **Salmond RJ**, Filby A, Qureshi I, Caserta S, Zamoyska R. T-cell receptor proximal signaling via the Src-family kinases, Lck and Fyn, influences T-cell activation, differentiation, and tolerance. *Immunol Rev* 2009; **228**: 9-22

146 **Tibaldi E**, Venerando A, Zonta F, Bidoia C, Magrin E, Marin O, Toninello A, Bordin L, Martini V, Pagano MA, Brunati AM. Interaction between the SH3 domain of Src family kinases and the proline-rich motif of HTLV-1 p13: a novel mechanism underlying delivery of Src family kinases to mitochondria. *Biochem J* 2011; **439**: 505-516

147 **Andresen V**, Pise-Masison CA, Sinha-Datta U, Bellon M, Valeri V, Washington Parks R, Cecchinato V, Fukumoto R, Nicot C, Franchini G. Suppression of HTLV-1 replication by Tax-mediated rerouting of the p13 viral protein to nuclear speckles. *Blood* 2011; **118**: 1549-1559

148 **Silic-Benussi M**, Biasiotto R, Andresen V, Franchini G, D' Agostino DM, Ciminale V. HTLV-1 p13, a small protein with a busy agenda. *Mol Aspects Med* 2010; **31**: 350-358

149 **Li S**. Src kinase signaling in leukaemia. *Int J Biochem Cell Biol* 2007; **39**: 1483-1488

150 **Zhao TM**, Robinson MA, Sawasdikosol S, Simpson RM, Kindt TJ. Variation in HTLV-I sequences from rabbit cell lines with diverse in vivo effects. *Virology* 1993; **195**: 271-274

151 **Mahana W**, Zhao TM, Teller R, Robinson MA, Kindt TJ. Genes in the pX region of human T cell leukemia virus I influence Vav phosphorylation in T cells. *Proc Natl Acad Sci USA* 1998; **95**: 1782-1787

152 **Baydoun HH**, Pancewicz J, Bai X, Nicot C. HTLV-I p30 inhibits multiple S phase entry checkpoints, decreases cyclin E-CDK2 interactions and delays cell cycle progression. *Mol Cancer* 2010; **9**: 302

153 **Datta A**, Silverman L, Phipps AJ, Hiraragi H, Ratner L, Lairmore MD. Human T-lymphotropic virus type-1 p30 alters cell cycle G2 regulation of T lymphocytes to enhance cell survival. *Retrovirology* 2007; **4**: 49

154 **Lemasson I**, Lewis MR, Polakowski N, Hivin P, Cavanagh MH, Thébault S, Barbeau B, Nyborg JK, Mesnard JM. Human T-cell leukemia virus type 1 (HTLV-1) bZIP protein interacts with the cellular transcription factor CREB to inhibit HTLV-1 transcription. *J Virol* 2007; **81**: 1543-1553

155 **Clerc I**, Polakowski N, André-Arpin C, Cook P, Barbeau B, Mesnard JM, Lemasson I. An interaction between the human T cell leukemia virus type 1 basic leucine zipper factor (HBZ) and the KIX domain of p300/CBP contributes to the down-regulation of tax-dependent viral transcription by HBZ. *J Biol Chem* 2008; **283**: 23903-23913

156 **Isono O**, Ohshima T, Saeki Y, Matsumoto J, Hijikata M, Tanaka K, Shimotohno K. Human T-cell leukemia virus type 1 HBZ protein bypasses the targeting function of ubiquitination. *J Biol Chem* 2008; **283**: 34273-34282

157 **Matsuoka M**, Green PL. The HBZ gene, a key player in HTLV-1 pathogenesis. *Retrovirology* 2009; **6**: 71

158 **Hicke L**. Protein regulation by monoubiquitin. *Nat Rev Mol Cell Biol* 2001; **2**: 195-201

159 **Chen BJ**, Lamb RA. Mechanisms for enveloped virus budding: can some viruses do without an ESCRT? *Virology* 2008; **372**: 221-232

160 **Rauch S**, Martin-Serrano J. Multiple interactions between the ESCRT machinery and arrestin-related proteins: implications for PPXY-dependent budding. *J Virol* 2011; **85**: 3546-3556

161 **Heidecker G**, Lloyd PA, Soheilian F, Nagashima K, Derse D. The role of WWP1-Gag interaction and Gag ubiquitination in assembly and release of human T-cell leukemia virus type 1. *J Virol* 2007; **81**: 9769-9777

162 **Blot V**, Perugi F, Gay B, Prévost MC, Briant L, Tangy F, Abriel H, Staub O, Dokhélar MC, Pique C. Nedd4.1-mediated ubiquitination and subsequent recruitment of Tsg101 ensure HTLV-1 Gag trafficking towards the multivesicular body pathway prior to virus budding. *J Cell Sci* 2004; **117**: 2357-2367

163 **Blot V**, Delamarre L, Perugi F, Pham D, Bénichou S, Benarous R, Hanada T, Chishti AH, Dokhélar MC, Pique C. Human Dlg protein binds to the envelope glycoproteins of human T-cell leukemia virus type 1 and regulates envelope mediated cell-cell fusion in T lymphocytes. *J Cell Sci* 2004; **117**: 3983-3993

S- Editor Zhang SS **L- Editor** Roemmele A **E- Editor** Zheng XM

World Journal of Virology

Online Submissions: http://www.wjgnet.com/esps/
wjv@wjgnet.com
www.wjgnet.com

World J Virology 2012 August 12; 1(4): I
ISSN 2220-3249 (online)

Acknowledgments to reviewers of *World Journal of Virology*

We acknowledge our sincere thanks to our reviewers. Many reviewers have contributed their expertise and time to the peer review, a critical process to ensure the quality of our World Series Journals. Both the editors of the journals and authors of the manuscripts submitted to the journals are grateful to the following reviewers for reviewing the articles (either published or rejected) over the past period of time.

Alexander A Bukreyev, PhD, Professor, Galveston National Laboratory, Center for Biodefense and Emerging Infectious Diseases, Department of Pathology, 301 University Boulevard, University of Texas Medical Branch, Galveston, TX 77555-0609, United States

Anda Baicus, MD, PhD, Lecturer, Senior Scientific Researcher, Head of the Enteric Viral infections Laboratory, Cantacuzino National Institute of Research-Development for Microbiology and Immunology, Spl. Independentei 103, Sect.5, 050096, Bucharest, Romania

Eiichi N Kodama, MD, PhD, Assistant Professor, Department of Internal Medicine, Division of Emerging Infectious Diseases, Tohoku University School of Medicine, Building 1 Room 515, 2-1 Seiryo, Aoba-ku, Sendai 980-8575, Japan

Gualtiero Alvisi, PhD, Department of Infectious Diseases, Heidelberg University, INF345, Heidelberg 69121, Germany

Hailong Guo, DVM, PhD, David H. Smith Center for Vaccine Biology and Immunology, University of Rochester Medical Center , 601 Elmwood Avenue, Box 609, Rochester , NY 14642, United States

Hsin-Wei Chen, Associate Investigator, Vaccine Research and Development Center, National Health Research Institutes, No. 35, Keyan Road, Zhunan Town, Miaoli County 350, Taiwan, China

Ian M Mackay, PhD, Qpid Laboratory, Sir Albert Sakzewski Virus Research Centre, Royal Children's Hospital, Herston Road, Herston Q4029, Australia

Jayanta Bhattacharya, PhD, Scientist D, Department of Molecular Virology, National AIDS Research Institute, 73G, MIDC, Bhosari, Pune 411026, India

José A Melero, PhD, Director, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, 28220 Madrid, Spain

Luis Menéndez-Arias, Professor, Centro de Biología Molecular "Severo Ochoa", c/Nicolás Cabrera, 1, Campus de Cantoblanco, 28049 Madrid, Spain

Majid Laassri, PhD, Staff Scientist, Office of Vaccines Research and Review, CBER, FDA, 1401 Rockville Pike, HFM-470, Rockville, MD 20852-1448, United States

Maurizia Debiaggi, PhD, Department of Morphological Sciences, Microbiology Unit, University of Pavia, via Brambilla 74, 27100 Pavia, Italy

Pablo Daniel Ghiringhelli, PhD, Director Ligbcm-Avi, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Roque Saenz Peña 352, B1876BXD Bernal, Argentina

Parin Chaivisuthangkura, PhD, Associate Professor, Department of Biology, Faculty of Science, Srinakharinwirot University, Sukhumvit 23, Wattana, Bangkok 10110, Thailand

Roberto Manfredi, MD, Associate Professor of Infectious Diseases, University of Bologna, c/o Infectious Diseases, S. Orsola Hospital, Via Massarenti 11, I-40138 Bologna, Italy

Preeti Bharaj, PhD, Diabetes and Obesity, Sanford-Burnham MRI, 6400 Sanger Road, Orlando, FL 32827, United States

Samia Ahmed Kamal, PhD, Virology Department, Animal Health Research Institute, Nadi Al- Said street, Beside Ministry of Agriculture, Dokki, Cairo, Egypt

Seyed Mojtaba Ghiasi, Assistant Professor, Arboviruses and Viral Hemorrhagic Fevers Laboratory, Pasteur Institute of Iran, No. 69, 12 Farvardin St, Pasteur Ave, Pasteur Sq, Tehran 1316943551, Iran

Sibnarayan Datta, PhD, Scientist, Biotechnology Division, Defence Research Laboratory, Post Bag no.2, Tezpur, Assam 784 001, India

World Journal of Virology

Online Submissions: http://www.wjgnet.com/esps/
wjv@wjgnet.com
www.wjgnet.com

World J Virol 2012 August 12; 1(4): I
ISSN 2220-3249 (online)

Events Calendar 2012

January 13-14, 2012
Innovation in Severe Acute Respiratory Infections
Sitges, Spain

January 20, 2012
Exploiting Bacteriophages for Bioscience, Biotechnology and Medicine
Welwyn Garden City,
United Kingdom

January 20-22, 2012
International Science Symposium on HIV and Infectious Diseases
Chennai, India

February 26-29, 2012
10th American Society for Microbiology Biodefense and Emerging Diseases Research Meeting
Washington, DC, United States

March 11-15, 2012
8th International Symposium on Pneumococci and Pneumococcal Diseases
Foz de Iguaçu City, Brazil

March 12-16, 2012
Vaccine World Summit India
Hyderabad, India

March 14-17, 2012
22nd Annual Meeting of the German Society for Virology
Essen, Germany

March 21-26, 2012
2012 HIV Vaccines
Keystone, CO, United States

March 21-26, 2012
Viral Immunity and Host Gene Influence
Keystone, CO, United States

March 26-31, 2012
Cell Biology of Virus Entry, Replication and Pathogenesis
Whistler, BC, Canada

March 26-31, 2012
Frontiers in HIV Pathogenesis, Therapy and Eradication
Whistler, BC, Canada

April 20-21, 2012
2012 Molecular Virology Workshop
Daytona Beach, FL, United States

April 22-25, 2012
2012 28th Clinical Virology Symposium
Daytona Beach, FL, United States

April 24-27, 2012
European Molecular Biology Organization Workshop - Antigen presentation and processing
Amsterdam, Netherlands

May 19-22, 2012
European Molecular Biology Organization and European Molecular Biology Laboratory Symposium - New perspectives on immunity to infection
Heidelberg, Germany

June 11-16, 2012
Antiviral RNAi: From Molecular Biology Towards Applications
Pultusk, Poland

June 28-29, 2012
7th International Workshop on Hepatitis C - Resistance and New Compounds
Cambridge, MA, United States

July 16-20, 2012
European Molecular Biology Organization Conference Series - Viruses of microbes: From exploration to applications in the -omics era
Brussels, Belgium

July 19–20, 2012
7th International Workshop on HIV Transmission - Principles of Intervention
Washington, DC, United States

July 21-25, 2012
31st Annual Meeting of American Society for Virology
Madison, WI, United States

July 30 - August 1, 2012
3rd Annual Symposia of Hepatitis Virus
Guangzhou, China

August 20-22, 2012
2nd World Congress on Virology
Las Vegas, NV, United States

September 7-9, 2012
Viral Hepatitis Congress 2012
The Johann Wolfgang Goethe University, Frankfurt, Germany

October 18-20, 2012
2nd World Congress on Controversies in the Management of Viral Hepatitis (C-Hep)
Berlin, Germany

November 28 -December 1, 2012
6th Asian Congress of Paediatric Infectious Diseases
Colombo, Sri Lanka

World Journal of Virology

Online Submissions: http://www.wjgnet.com/esps/
wjv@wjgnet.com
www.wjgnet.com

World J Virol 2012 August 12; 1(4): I-V
ISSN 2220-3249 (online)

GENERAL INFORMATION

World Journal of Virology (*World J Virol*, *WJV*, online ISSN 2220-3249, DOI: 10.5501) is a bimonthly peer-reviewed, online, open-access (OA), journal supported by an editorial board consisting of 137 experts in virology from 41 countries.

The biggest advantage of the OA model is that it provides free, full-text articles in PDF and other formats for experts and the public without registration, which eliminates the obstacle that traditional journals possess and usually delays the speed of the propagation and communication of scientific research results. The open access model has been proven to be a true approach that may achieve the ultimate goal of the journals, i.e. the maximization of the value to the readers, authors and society.

Maximization of personal benefits

The role of academic journals is to exhibit the scientific levels of a country, a university, a center, a department, and even a scientist, and build an important bridge for communication between scientists and the public. As we all know, the significance of the publication of scientific articles lies not only in disseminating and communicating innovative scientific achievements and academic views, as well as promoting the application of scientific achievements, but also in formally recognizing the "priority" and "copyright" of innovative achievements published, as well as evaluating research performance and academic levels. So, to realize these desired attributes of *WJV* and create a well-recognized journal, the following four types of personal benefits should be maximized. The maximization of personal benefits refers to the pursuit of the maximum personal benefits in a well-considered optimal manner without violation of the laws, ethical rules and the benefits of others. (1) Maximization of the benefits of editorial board members: The primary task of editorial board members is to give a peer review of an unpublished scientific article via online office system to evaluate its innovativeness, scientific and practical values and determine whether it should be published or not. During peer review, editorial board members can also obtain cutting-edge information in that field at first hand. As leaders in their field, they have priority to be invited to write articles and publish commentary articles. We will put peer reviewers' names and affiliations along with the article they reviewed in the journal to acknowledge their contribution; (2) Maximization of the benefits of authors: Since *WJV* is an OA journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from *WJV* official website, thereby realizing the goals and significance of the communication between authors and peers as well as public reading; (3) Maximization of the benefits of readers: Readers can read or use, free of charge, high-quality peer-reviewed articles without any limits, and cite the arguments, viewpoints, concepts, theories, methods, results, conclusion or facts and data of pertinent literature so as to validate the innovativeness, scientific and practical values of their own research achievements, thus ensuring that their articles have novel arguments or viewpoints, solid evidence and correct conclusion; and (4) Maximization of the benefits of employees: It is an iron law that a first-class journal is unable to exist without first-class editors, and only first-class editors can create a first-class academic journal. We insist on strengthening our team cultivation and construction so that every employee, in an open, fair and transparent environment, could contribute their wisdom to edit and publish high-quality articles, thereby realizing the maximization of the personal benefits of editorial board members, authors and readers, and yielding the greatest social and economic benefits.

Aims and scope

WJV aims to report rapidly new theories, methods and techniques for prevention, diagnosis, treatment, rehabilitation and nursing in the field of virology. *WJV* covers topics concerning arboviral infections, bronchiolitis, central nervous system viral diseases, DNA virus infections, encephalitis, eye infections, fatigue syndrome, hepatitis, meningitis, opportunistic infections, pneumonia, RNA virus infections, sexually transmitted diseases, skin diseases, slow virus diseases, tumor virus infections, viremia, zoonoses, and virology-related traditional medicine, and integrated Chinese and Western medicine. The journal also publishes original articles and reviews that report the results of virology-related applied and basic research in fields such as immunology, physiopathology, cell biology, pharmacology, medical genetics, and pharmacology of Chinese herbs.

Columns

The columns in the issues of *WJV* will include: (1) Editorial: To introduce and comment on the substantial advance and its importance in the fast-developing areas; (2) Frontier: To review the most representative achievements and comment on the current research status in the important fields, and propose directions for the future research; (3) Topic Highlight: This column consists of three formats, including (A) 10 invited review articles on a hot topic, (B) a commentary on common issues of this hot topic, and (C) a commentary on the 10 individual articles; (4) Observation: To update the development of old and new questions, highlight unsolved problems, and provide strategies on how to solve the questions; (5) Guidelines for Clinical Practice: To provide guidelines for clinical diagnosis and treatment; (6) Review: To systemically review the most representative progress and unsolved problems in the major scientific disciplines, comment on the current research status, and make suggestions on the future work; (7) Original Articles: To originally report the innovative and valuable findings in virology; (8) Brief Articles: To briefly report the novel and innovative findings in virology; (9) Case Report: To report a rare or typical case; (10) Letters to the Editor: To discuss and make reply to the contributions published in *WJV*, or to introduce and comment on a controversial issue of general interest; (11) Book Reviews: To introduce and comment on quality monographs of virology; and (12) Guidelines: To introduce consensuses and guidelines reached by international and national academic authorities worldwide on the research in virology.

Name of journal

World Journal of Virology

ISSN

ISSN 2220-3249 (online)

Editor-in-Chief

Ling Lu, MD, PhD, Department of Pathology and Laboratory

Medicine, University of Kansas Medical Center, Kansas City, 3901 Rainbow Blvd, WHE 3020, KS 66160, United States

Editorial office
World Journal of Virology
Editorial Department: Room 903, Building D,
Ocean International Center,
No. 62 Dongsihuan Zhonglu,
Chaoyang District, Beijing 100025, China
E-mail: wjv@wjgnet.com
http://www.wjgnet.com
Telephone: +86-10-85381891
Fax: +86-10-85381893

Indexed and abstracted in
Digital Object Identifier

Published by
Baishideng Publishing Group Co., Limited

SPECIAL STATEMENT

All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

Biostatistical editing
Statistical review is performed after peer review. We invite an expert in Biomedical Statistics to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, Ridit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance, *etc*. The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

Conflict-of-interest statement
In the interests of transparency and to help reviewers assess any potential bias, *WJV* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work. Referees are also asked to indicate any potential conflict they might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

Sample wording: [Name of individual] has received fees for serving as a speaker, a consultant and an advisory board member for [names of organizations], and has received research funding from [names of organization]. [Name of individual] is an employee of [name of organization]. [Name of individual] owns stocks and shares in [name of organization]. [Name of individual] owns patent [patent identification and brief description].

Statement of informed consent
Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

Statement of human and animal rights
When reporting the results from experiments, authors should follow the highest standards and the trial should conform to Good Clinical Practice (for example, US Food and Drug Administration Good Clinical Practice in FDA-Regulated Clinical Trials; UK Medicines Research Council Guidelines for Good Clinical Practice in Clinical Trials) and/or the World Medical Association Declaration of Helsinki. Generally, we suggest authors follow the lead investigator's national standard. If doubt exists whether the research was conducted in accordance with the above standards, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Baishideng Publishing Group Co., Limited, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the ICMJE to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www.clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be returned to the author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

Online submissions
Manuscripts should be submitted through the Online Submission System at: http://www.wjgnet.com/esps/. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/2220-3249/g_info_20100722180909.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to wjv@wjgnet.com, or by telephone: +86-10-85381891. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page

Title: Title should be less than 12 words.

Running title: A short running title of less than 6 words should be provided.

Authorship: Authorship credit should be in accordance with the standard proposed by ICMJE, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Institution: Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece

Author contributions: The format of this section should be: Author contributions: Wang CL and Liang L contributed equally to this work; Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; and Wang CL, Liang L and Fu JF wrote the paper.

Supportive foundations: The complete name and number of supportive foundations should be provided, e.g. Supported by National Natural Science Foundation of China, No. 30224801

Correspondence to: Only one corresponding address should be provided. Author names should be given first, then author title, affiliation, the complete name of institution, city, postcode, province, country, and email. All the letters in the email should be in lower case. A space interval should be inserted between country name and email address. For example, Montgomery Bissell, MD, Professor of Medicine, Chief, Liver Center, Gastroenterology Division, University of California, Box 0538, San Francisco, CA 94143, United States. montgomery.bissell@ucsf.edu

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g. Telephone: +86-10-85381891 Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision for acceptance is made only when at least two experts recommend an article for publication. Reviewers for accepted manuscripts are acknowledged in each manuscript, and reviewers of articles which were not accepted will be acknowledged at the end of each issue. To ensure the quality of the articles published in *WJV*, reviewers of accepted manuscripts will be announced by publishing the name, title/position and institution of the reviewer in the footnote accompanying the printed article. For example, reviewers: Professor Jing-Yuan Fang, Shanghai Institute of Digestive Disease, Shanghai, Affiliated Renji Hospital, Medical Faculty, Shanghai Jiaotong University, Shanghai, China; Professor Xin-Wei Han, Department of Radiology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan Province, China; and Professor Anren Kuang, Department of Nuclear Medicine, Huaxi Hospital, Sichuan University, Chengdu, Sichuan Province, China.

Abstract

There are unstructured abstracts (no less than 256 words) and structured abstracts (no less than 480). The specific requirements for structured abstracts are as follows:

An informative, structured abstracts of no less than 480 words should accompany each manuscript. Abstracts for original contributions should be structured into the following sections. AIM (no more than 20 words): Only the purpose should be included. Please write the aim as the form of "To investigate/study/…; MATERIALS AND METHODS (no less than 140 words); RESULTS (no less than 294 words): You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g. 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$; CONCLUSION (no more than 26 words).

Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Text

For articles of these sections, original articles and brief articles, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both. The main text format of these sections, editorial, topic highlight, case report, letters to the editors, can be found at: http://www.wjgnet.com/2220-3249/g_info_20100725072755.htm.

Illustrations

Figures should be numbered as 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Figures should be either Photoshop or Illustrator files (in tiff, eps, jpeg formats) at high-resolution. Examples can be found at: http://www.wjgnet.com/1007-9327/13/4520.pdf; http://www.wjgnet.com/1007-9327/13/4554.pdf; http://www.wjgnet.com/1007-9327/13/4891.pdf; http://www.wjgnet.com/1007-9327/13/4986.pdf; http://www.wjgnet.com/1007-9327/13/4498.pdf. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: …*etc.* It is our principle to publish high resolution-figures for the printed and E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. $^{a}P < 0.05$, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of *P* values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of *P* values can be expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc.*, in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that..."

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

PMID and DOI

Pleased provide PubMed citation numbers to the reference list, e.g. PMID and DOI, which can be found at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed and http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in E-version of this journal.

Style for journal references

Authors: the name of the first author should be typed in bold-faced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634 DOI: 10.3748/wjg.13.5396].

Style for book references

Authors: the name of the first author should be typed in bold-faced letters. The surname of all authors should be typed with the initial letter capitalized, followed by their abbreviated middle and first initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pres-

sure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/2220-3249/g_info_20100725073806.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H. pylori*, *E coli*, *etc.*

Examples for paper writing

Editorial: http://www.wjgnet.com/2220-3249/g_info_20100725071851.htm

Frontier: http://www.wjgnet.com/2220-3249/g_info_20100725071932.htm

Topic highlight: http://www.wjgnet.com/2220-3249/g_info_20100725072121.htm

Observation: http://www.wjgnet.com/2220-3249/g_info_20100725072232.htm

Guidelines for basic research: http://www.wjgnet.com/2220-3249/g_info_20100725072344.htm

Guidelines for clinical practice: http://www.wjgnet.com/2220-3249/g_info_20100725072543.htm

Review: http://www.wjgnet.com/2220-3249/g_info_20100725072656.htm

Original articles: http://www.wjgnet.com/2220-3249/g_info_20100725072755.htm

Brief articles: http://www.wjgnet.com/2220-3249/g_info_20100725072920.htm

Case report: http://www.wjgnet.com/2220-3249/g_info_20100725073015.htm

Letters to the editor: http://www.wjgnet.com/2220-3249/g_info_20100725073136.htm

Book reviews: http://www.wjgnet.com/2220-3249/g_info_20100725073214.htm

Guidelines: http://www.wjgnet.com/2220-3249/g_info_20100725073300.htm

SUBMISSION OF THE REVISED MANUSCRIPTS AFTER ACCEPTED

Please revise your article according to the revision policies of *WJV*. The revised version including manuscript and high-resolution image figures (if any) should be re-submitted online (http://www.wjgnet.com/2220-3249office/). The author should send the copyright transfer letter, responses to the reviewers, English language Grade B certificate (for non-native speakers of English) and final manuscript checklist to wjv@wjgnet.com.

Language evaluation

The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; and (4) Grade D: rejected. Revised articles should reach Grade A or B.

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/2220-3249/g_info_20100725073726.htm.

Responses to reviewers

Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/2220-3249/g_info_20100725073445.htm.

Proof of financial support

For paper supported by a foundation, authors should provide a copy of the document and serial number of the foundation.

Links to documents related to the manuscript

WJV will be initiating a platform to promote dynamic interactions between the editors, peer reviewers, readers and authors. After a manuscript is published online, links to the PDF version of the submitted manuscript, the peer-reviewers' report and the revised manuscript will be put on-line. Readers can make comments on the peer reviewer's report, authors' responses to peer reviewers, and the revised manuscript. We hope that authors will benefit from this feedback and be able to revise the manuscript accordingly in a timely manner.

Science news releases

Authors of accepted manuscripts are suggested to write a science news item to promote their articles. The news will be released rapidly at EurekAlert/AAAS (http://www.eurekalert.org). The title for news items should be less than 90 characters; the summary should be less than 75 words; and main body less than 500 words. Science news items should be lawful, ethical, and strictly based on your original content with an attractive title and interesting pictures.

Publication fee

WJV is an international, peer-reviewed, Open-Access, online journal. Articles published by this journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license. Authors of accepted articles must pay a publication fee. Publication fee: 600 USD per article. Editorial, topic highlights, book reviews and letters to the editor are published free of charge.