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EDITORIAL

# Intersecting pathways in inflammation and cancer: Hepatocellular carcinoma as a paradigm

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## Abstract

Viral infection and chemical carcinogens trigger somatic changes resulting in activation of oncogenes during tumor initiation in the development of cancer. However, a critical interaction resides in the synergism between these somatic changes and an inflamed tumor microenvironment where myeloid and hematopoietic cells are subverted to enhance tumor progression. The causative molecular mechanisms leading to the development of hepatocellular cancer remain incompletely understood but appear to result from multiple factors related to direct hepatocyte injury and the ensuing inflammatory changes mediated by the host response to tissue injury, DNA damage, repair of cellular damage, and chronic, repetitive injury. In this review, the molecular and cellular changes that regulate inflammation and tissue repair will be compared to the activated local tumor microenvironment. Cell-cell signaling within this microenvironment that enhances tumor progression and inhibits anti-tumor immunity will be discussed



**Key words:** Hepatocellular cancer; Inflammation; Tumor microenvironment

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## INTRODUCTION

Cellular injury resulting in inflammation, tissue repair, and fibrosis has been hypothesized to play a functional role in cancer development since Virchow's original observations[1]. Key somatic changes caused by viral or chemical carcinogens establishing a "subthreshold neoplastic state" (initiation) is often supplemented by a subsequent stimulus from the local niche or microenvironment, such as chronic inflammation (promotion). These synergistic pathways complement each other to augment tumor invasiveness[2,3]. In certain types of cancers, inflammatory conditions are present before a malignant change occurs and investigators have sought to define a causal relationship between inflammation, innate immunity and cancer development. Certainly, epidemiological studies have shown that chronic inflammation predisposes individuals to various types of cancer including bladder, cervical, gastric, intestinal, esophageal, ovarian, prostate, liver, and thyroid tumors. Perhaps the most

convincing evidence is the fact that adoptive transfer of inflammatory cells from the local tumor environment or overexpression of inflammatory cytokines promotes tumor development[4]. Despite these observations, the fundamental mechanisms by which inflammation leads to cancer or supports the progression of cancer remain unclear.

In this editorial, we will discuss the critical components of the inflammatory milieu or tumor microenvironment (TME) that promote a neoplastic and/or metastatic phenotype. The canonical physiology of inflammation will be examined within the context of cancer development highlighting similarities and differences between the two analogous processes. Finally, hepatitis and chronic liver cirrhosis will be discussed as a model of fibrogenesis and hepatic carcinogenesis with potential therapeutic targets.

## INFLAMMATION, TISSUE REPAIR, TUMOR PROGRESSION AND METASTASIS

Mantovani and colleagues defined the complementary oncogenic and inflammatory processes in cancer development as the "intrinsic" and "extrinsic" pathways[5]. The intrinsic pathway activates oncogenes and inhibits tumor suppressors by mutation, chromosomal rearrangement or amplification and drives transformation within targeted cells. Tumor cells generated in this fashion subsequently produce cytokines that recruit and populate the inflammatory TME. The extrinsic pathway derives from inflammatory or infectious conditions that amplify the cancer risk (e.g., inflammatory bowel disease, hepatitis, *Helicobacter pylori*). These two mutually dependent pathways eventually converge, appropriating necessary components and signals from the other while also supplying reciprocally useful building blocks to fuel transformation and metastasis in a cooperated fashion. Oncogenes that can produce signals for inflammatory cell recruitment include the RET proto-oncogene in papillary thyroid cancer, K-RAS in pancreatic cancer, and BRAF-MAPK in melanoma[5]. It is no coincidence that inflammation and wound healing physiology parallels the tissue remodeling processes that occur in cancer progression. Dvorak[6] recognized that the composition of the tumor stroma strongly resembles the granulation tissue of healing skin wounds. These important, essential inflammatory cascades promote cell proliferation, migration, invasion through the extracellular matrix, angiogenesis, and ultimately provide the necessary components for host tissue repair and survival. In many types of cancer, these attributes can be subverted by nascent tumor cells as tools for cancer progression and metastasis.

The steps of the inflammatory cascade associated with tissue repair are well characterized.Tissue injury created by toxins, infection, or a chronic inflammatory stimulus results in a host response focused on recruiting cells that initiate healing (Figure 1). Critical members of this microenvironment include neutrophils, monocytes, macrophages, mast cells, dendritic cells, fibroblasts and endothelial cells. The wound healing process often involves partially overlapping phases: blood clotting, inflammation, new tissue formation, and tissue remodeling[7] with key cell types recruited to the niche during specific phases. Important pro-inflammatory signals produced during this cascade include IL-1β, IL-6, IL-23, TNF-α, and TGF-β1. Activation of the selectin family of adhesion molecules (L-, P-, and E-selectin) facilitates leukocyte "rolling" along the injured vascular endothelium, activating integrin binding and immobilization (α4β1 and α4β7 binding to VCAM-1 and MadCAM-1), and ultimately transmigration through the endothelium into the site of injury[7]. Release of cytokines, chemokines, and prostaglandins to recruit additional inflammatory cells, the production of reactive oxygen species (ROS) to destroy infectious vectors, the generation of pro-angiogenic factors, and modulation of apoptosis represent other essential,activated functions.

Physiological inflammation is often self-limiting through downstream release of anti-inflammatory regulators (IL-10, IL-11, IL-13) which temper the pro-inflammatory cascade. However, cancer-associated inflammation can often persist, or be driven without regulation,to elicit pathologically persistent signals for cellular proliferation, migration, basement membrane invasion and angiogenesis. In this context,tumors have been comparatively described as "wounds that do not heal"[6]. The key cells residing in the TME are the same constituents that facilitate wound healing and inflammation as described above. However, the tumor-associated cells recruited often display altered functions that lend themselves to cancer development. This alteration in function derives from the upregulated expression of pro-tumor cytokines. For example, dendritic cells in neoplastic infiltrates are regulated by tumor-derived granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-4 and are frequently immature, less effective at capturing antigens, and defective in T-cell stimulatory capacity[2]. IL-10 released into the TME is a potent inhibitor of dendritic cell activation and differentiation allowing evasion of host adaptive immunity[5]. Similar processes occur in tumor-associated macrophages (TAMs) which produce a number of potent angiogenic and lymphangiogenic growth factors, cytokines and proteases that mediate neoplastic progression. For example, in human cervical carcinogenesis, TAMs express VEGF-C, VEGF-D, and VEGF receptor-3, to facilitate angiogenesis[8]. In a murine mammary cancer metastasis model, CSF-1 regulates tumor growth by supporting and cultivating the TME. In CSF-1$^{-/-}$ mice, advanced mammary tumors and pulmonary metastases fail to develop due to decreased TAMs recruitment into the neoplastic tissue[9]. CSF-1 has been shown to promote progression of mammary tumors to malignancy as replacement of transgenic CSF-1 into mammary epitheli-

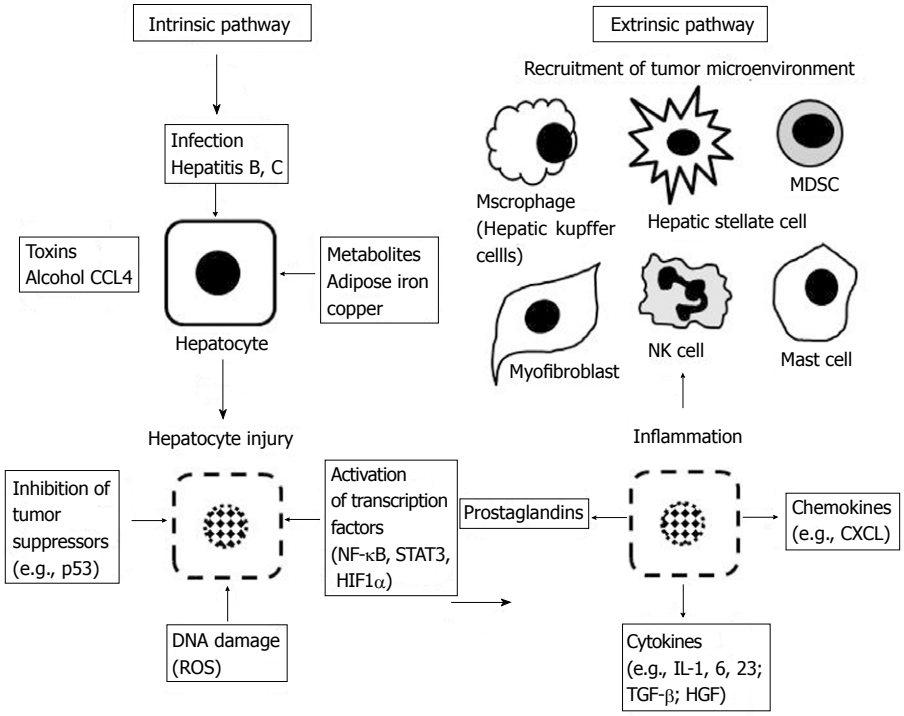


**Figure 1 The intrinsic and extrinsic pathways combine to create a local microenvironment around the injured and transformed hepatocyte to augment tumor promoting mechanisms**. ROS: Reactive oxygen species; HIF1α: Hypoxia inducible factor 1 alpha; NK cell: Natural killer cell; MDSC: Myeloid derived suppressor cell; IL: Interleukin; TGF-β: Transforming growth factor Beta; HGF: Hepatocyte growth factor; NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; CXCL: Chemokine ligand; STAT3: Signal transducer and activator of transcription 3.

um restores macrophage recruitment, primary tumor development, and metastatic potential[9]. A powerful stimulus for tumor progression within the TME includes the ROS derived from infiltrating leukocytes. In the presence of chronic inflammation and repetitive injury, leukocytes and other phagocytic cells induce DNA damage in proliferating cells through the generation of reactive oxygen and nitrogen species such as peroxynitrite. Irreversible DNA mutations generated by these reactive species can provide the critical trigger for neoplastic transformation. In addition to these mechanisms, the inhibition of tumor-suppressor pathways represent yet another strategy for promoting tumor growth. Macrophage migration inhibitory factor (MIF) released from TAMs and T lymphocytes is a potent cytokine that suppresses *p53* transcriptional activity. MIF released into the TME creates a niche with a deficient response to DNA damage[10]. TAMs will be diverted into the M2 phenotype in human tumors so that macrophage functions will be focused on promoting tumor growth, remodeling tissues, promoting angiogenesis, and suppressing adaptive immunity[11,12]. Another class of cells that are recruited to the TME include the myeloid-derived suppressor cells (MDSCs). These cells are abundant in tumors and strongly inhibit anti-tumor immunity[7]. MDSCs represent an immature population of myeloid cells that inhibit both innate and adaptive immunity and are present in cancer patients and in experimental animals with sizable tumor burden[13]. Although no definitive molecular characterization exists, many investigators have found human MDSCs to express CD33, CD11b and CD15 cell surface markers[13]. MDSC inhibition of anti-tumor immunity is mediated by suppression of CD4+ T-cells[14], inducing T regulatory cells[15], by down-regulating macrophage production of the type 1 cytokine, IL-12[16], and potentially suppressing natural killer cell cytotoxicity[17]. In hepatocellular carcinoma models, trafficking and accumulation of MDSCs appears to be gp130 dependent and downregulation of NK cell cytokine production to be NKp30 dependent[18]. Recent studies have also focused on the myofibroblast as another cell type that is commonly found in wounds and in the TME and has been implicated in tumor progression. The presence of large numbers of fibroblasts and myofibroblasts is a hallmark of cancer with many tumors producing a desmoplastic response[7]. Although tumor fibroblasts can be derived from the stroma surrounding tumors, there is evidence to suggest that cells recruited from the bone-marrow also 'home in' on the TME[19]. Auto- and paracrine PDGF and TGF-β dependent signaling centered on the myofibroblast is considered fundamental to tumor progression, the development of epithelial-mesenchymal transition (EMT),and generation of cancer stem cells (CSCs). CSCs exhibit a $CD44^{high}$/$CD24^{low}$antigenic phenotype, demonstrate upregulation of the mesenchymal markers and the transcription factors, N-cadherin, fibronectin, vimentin, FOXC2, SIP1, Hedgehog (Hh), Snail, and Twist, and possess self-renewal capability enabling CSCs to exit tissue reservoirs,

enter and survive in the circulation, and exit into secondary tissue sites ("stemness")[20]. In the liver, cancer-associated fibroblasts are important contributors to the TME[21] and their precise origin continue to be unclear with a variety of hepatic cells able to generate stem-cell characteristics including hepatocytes, oval cells/hepatic progenitor cells, and bone marrow-derived cells[22].

## HEPATIC FIBROGENESIS AND TUMOR PROGRESSION

Constituents of the cancer-associated inflammatory stroma vary between different tumor types suggesting that TMEs can be disease-specific. Distinguishing mechanistic pathways that are conserved versus pathways that are tissue- and tumor- specific is important. Friedman and colleagues described the difference between "core" and "non-core" pathways leading to end-stage inflammation or fibrosis with "core" pathways maintaining a dominant role through different organ systems, processes, and species[23]. Non-core pathways may regulate core pathways, but are not necessarily fundamental to the fibrotic process, and may be specific to one model system without correlation to other disease states. Discovery of universal anti-fibrotic cures may require specific attention to core pathways. However, such integral proteins and signals (e.g., TGF-β) may be required in normal tissue function and may not be appropriate targets for inhibition. In this context, regulatory signaling pathways that are tissue-specific or even disease/pathology-specific may provide realistic targets that can be candidates for therapeutic intervention.

Hepatic fibrosis is a reversible wound-healing response to liver injury and is characterized by inflammation, accumulation of extracellular matrix (ECM), and ultimately scarring, as described above. If the injury is self-limiting, the inflammatory changes are transient and the liver tissue is restored to its normal configuration. However, when the injury or the resultant inflammatory response is persistent, the liver architecture is irreversibly transformed leading to progressive fibrosis and cirrhosis. Agents that injure the liver in such a way include toxins (CCL4, alcohol, or bile from biliary stasis), chronic infections (hepatitis B, hepatitis C), or remodeling processes (adipose tissue in non-alcoholic fatty liver disease). Chemical toxicity, viral infection, and metabolic derangements damage hepatocytes and this injury triggers a cascade designed to contain the injury by removing or repairing damaged cells, defense against further infection or injury, tissue regeneration and repair. Chronic inflammation due to repetitive injury (toxin) or inability to remove the offending agent (viral infection) results in a deranged, decompensated response (Figure 1). The liver is composed of constituent cells capable of mounting a robust inflammatory response. The space of Disse is a subendothelial space that separates the hepatic sinusoids from the hepatocytes. It contains a low density basal membrane-like matrix that is essential for maintaining the differentiated function of parenchymal cells but can become porous enough to enable metabolic exchange between the bloodstream and hepatocytes[24]. Inflammatory cells that can be activated to secrete cytokines and regulate ECM in this space include the hepatic macrophages (Kupffer cells), hepatic stellate cells (HSCs) (that can be activated to become fibroblasts), dendritic cells, natural killer cells, hepatocytes and cholangiocytes. Progression to cirrhosis leads to the formation of nodules of regenerative parenchyma surrounded by sheets of fibrotic septae. During chronic liver injury, extracellular matrix (ECM) deposition is upregulated. Interactions between the ECM and its surrounding cells then become mutually dependent with decorin and biglycan binding of TGF-β; fibronectin and laminin binding of TNF-α; and collagen binding to PDGF, HGF, and IL-2[24]. HSCs become activated from a quiescent vitamin A-rich cell to a contractile, highly fibrogenic, myofibroblast cell-type. Activated HSCs leads to upregulation of integrin receptors α2β1, α6β4, αvβ8, αvβ6, and α5β1, regulation of TGF-β1, PDGF, and Hedgehog (Hh) signaling pathways, expression of ADAMSTS-13, ADAMSTS-1, and secretion of collagenase-1 and -3, stromelysin-1 and -2, gelatinases, metalloelastases (MT-MMP1) and tissue inhibitor of metalloproteinase-1 (TIMP-1)[24] (Figure 2).

Accumulating evidence suggests that hepatocellular epithelial mesenchymal transition (EMT) plays a pivotal role in the dissemination of malignant hepatocytes during HCC progression[25]. Complete EMT often occurs in cancer cells as they lose cell-cell contacts, acquire a fibroblast-like morphology, and express mesenchymal marker proteins such as alpha smooth muscle actin (αSMA) and fibroblast-specific protein 1 (FSP1)[7], while shedding the epithelial cell markers cytokeratin and E-cadherin. This process often allows a firmly attached epithelial cell with appropriate apical-basal polarity to migrate into the interstitium and acquire characteristics of mesenchymal cells (highly motile, invasive, resistant to apoptosis, produce ECM, transmigrate through basement membrane, and intravasate into endothelium). Hepatic cancer cells that undergo EMT appear to acquire the properties of stem cells[20]. After liver injury occurs, the major trigger of EMT is the release of chemokines, MMPs, and other growth factors with PDGF, TGF-β and Hh occupying principal roles in this process (Figure 2).

### *TGF-β*

TGF-β is secreted by a variety of cell types and exists as the following isoforms: TGF-β1, TGF-β2, and TGF-β3. The predominant isoform in inflammation and fibrosis is TGF-β1 and signaling is mediated through the type Ⅱ receptor. PDGF receptors transduce their signals through the PI3K/Akt pathways while TGF-β mediates signaling through the Smad proteins. Inhibition of PDGF signaling decreases migration *in vitro* and efficient tumor suppression *in vivo* indicating that TGF-β mediated EMT of neoplastic hepatocytes is PDGF-dependent[25,26]. Activation of the type Ⅱ receptor results in di-

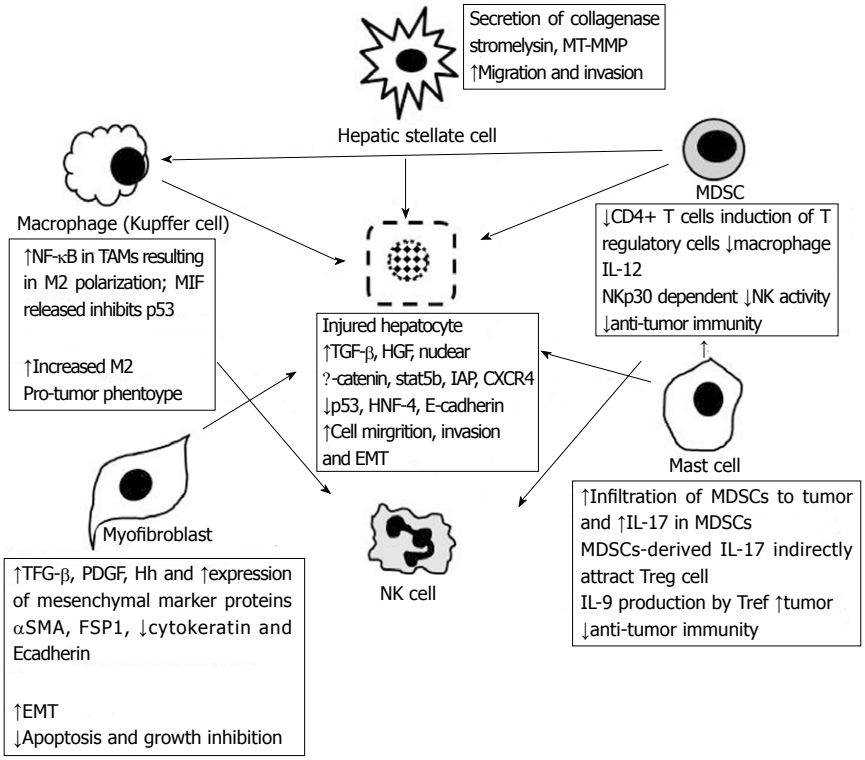


**Figure 2 The complex cellular network in the tumor microenvironment mediated by chemokines, cytokines, and cellular transcription factors.** NK cell: Natural killer cell; MDSC: Myeloid derived suppressor cell; IL: Interleukin; TGF- β:Transforming growth factor Beta; αSMA: Alpha smooth actin; FSP-1: Fibroblast specific protein; PDGF: Platelet derived growth factor; Hh: Hedgehog; NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; CXCL: Chemokine ligand; STAT3: Signal transducer and activator of transcription 3; Treg: T regulatory cells; MT-MMP: Membrane type matrix metalloproteinase; HNF-4: Hepatocyte nuclear factor-4; EMT: Epithelial-mesenchymal-transition; MIF: migration inhibitory factor.

merization with the type Ⅰ receptor and promotes binding to Smad2 and Smad3. The phosphorylated Smad2/Smad3 complex associates with Smad4 and activates transcription. TGF-? is a tumor suppressor protein that is frequently involved in tumor progression of human cancer and also in tumor-promoting inflammation. Many liver cell types, including HSCs, hepatocytes, and liver sinusoidal endothelial cells are regulated by TGF-?[27]. Interestingly, as in other cancer cell types, TGF-? has been shown to have a dual role in HCC with TGF-? displaying antitumor effects initially. For example, loss-of-function of TGF-? type Ⅱ receptor results in enhanced susceptibility to HCC, suggesting that TGF-? retains some tumor-suppressor functions[28]. Alternatively, transgenic mice with upregulated Smad7 expression restricted to hepatocytes demonstrate significantly diminished liver damage and fibrosis, demonstrating that TGF-? signaling in hepatocytes is required for fibrogenesis progression[29]. The significance of the dual nature of these effects is unclear but are reflected across other cancer cell types and suggest that the effects of TGF-? may be time-, and context-dependent. For example, inactivation of type Ⅱ TGF-? receptor in an animal model of breast carcinoma increases CXCL5- and CXCL12-mediated recruitment of myeloid-derived suppressor cells (MDSCs) which are potent suppressors of the adaptive immune response to tumors[5]. Smad7 activation or RNA interference against Smad4 decreases TGF-? signaling and attenuates the expression of pro-fibrotic genes[29,30]. However, hepatocytes isolated from livers exposed to high TGF-? *in vivo* demonstrate elongated, fibroblastoid hepatocytes expressing vimentin and collagen Ⅰ in comparison to healthy mouse livers[31].Other evidence for pro-tumor TGF-?-mediated downstream effects include studies by Morris *et al*[32] that show loss of TGF-? receptor type Ⅱ in the context of loss of *p53* decreased the incidence of HCC in a murine model of liver cancer. Cumulative evidence from the Fabregat group demonstrates that TGF-? signaling regulates seemingly contradictory processes in normal liver cells and in HCC. TGF-?-mediated growth inhibition and apoptosis (tumor-suppressor characteristics) occur in non-transformed human fetal hepatocytes while transdifferentiation into a mesenchymal-stem cell-like phenotype with increased expression of Snail, decreased E-cadherin expression, increased Vimentin and N-cadherin expression (pro-tumor) is also TGF-?-mediated[33]. Indeed, parallel experiments using siRNA-mediated down regulation of Snail showed that hepatocytes became sensitized to TGF-? mediated apoptosis and that Snail and induction of the EMT phenotype impairs TGF-? apoptosis in HCC cells[34].

### *Nuclear factor-kappa B*

Nuclear factor-kappa B (NF-?B) is an important regula-

tor of innate immunity,inflammation and also of tumor progression. NF-κB is activated downstream from Toll-like receptor (TLR)-MyD88 signaling and by signaling through the TNF-α and IL-1β pathways. NF-κB can also be activated as a result of genetic alterations (amplification, mutations, or deletions) in tumor cells. NF-κB activation is controlled by multiple factors and its transcriptional activity is linked to various inflammatory or pro-tumor states. Deficiency of TIR8, an inhibitor of Toll/IL-1 (TIR)-mediated NF-κB signaling, results in increased NF-κB activation with increased susceptibility to intestinal inflammation and carcinogenesis[35,36]. The causative role of NF-κB in inflammation and cancer is further supported by studies in TAMs where the p50 homodimers confer a pro-tumor phenotype through constitutively activated NF-κB[37]. Inhibition of IKK-α releases inhibition of maspin, a tumor suppressor, and reduces metastatic spread in malignant prostate epithelial cells[38]. Evidence also suggests that NF-κB determines the balance between pro- and anti-tumor effects. When NF-κB signaling is inhibited specifically in TAMs, they switch to a "classically" activated M1 phenotype (IL-12$^{high}$; IL-10$^{low}$) and TAMs become cytotoxic to tumor cells again[39]. Clearly, the mechanisms involved in NF-κB activation are complex and contradictory functions occur in liver physiology and hepatic carcinogenesis. In Mdr2-knockout mouse (a murine model of chronic inflammation induced-HCC), NF-κB inhibition with inducible IκB super-repressor resulted in decreased tumor progression[40]. In contrast, Maeda and colleagues demonstrated that IKK-β knockout in hepatocytes during an acute liver injury model with diethylnitrosamine (DEN) resulted in mice with an absence of chronic inflammation but increased hepatocarcinogenesis[41]. The timing and context of NF-κB activation or inhibition determines the associated phenotype.

### Signal transducer and activator of transcription

The signal transducer and activator of transcription (STAT) family of transcription factors also play a critical role in tumor and immune cells. STAT1 and STAT3 play a key role in liver fibrosis, antiviral defense, liver inflammation, and liver regeneration. STAT1 confers a protective effect and functions in down-regulating pro-fibrotic mechanisms in the liver by inhibiting HSC proliferation, suppression of β-PDGF receptor expression, inhibition of TGF-β/Smad3 signaling, and stimulation of NK cell cytotoxicity[42]. In these loss of function studies, STAT1$^{-/-}$ mice demonstrated accelerated CCL4-induced liver fibrosis and HSC proliferation.

STAT3 is one of the main signaling proteins activated by HGF and EGF receptors, and is involved in oncogenesis, inhibition of apoptosis, inhibition of dendritic cells, and increased evasion of the immune system[43]. Mice that lack STAT3 in the epidermis suffer from reduced wound re-epithelization, and are resistant to carcinogen-induced skin cancer development. Conversely, mice that overexpress a constitutively active form of STAT3 develop skin cancers with a shorter latency period[44].

### Chemokines

Tumor cells can regulate their chemokine expression profile to recruit inflammatory cells, but also use these factors to enhance tumor growth and expression[2]. The chemokines CXCL1, CXCL2, CXCL3, and CXCL8 have been extensively studied in melanoma and they play a role in the regulation of tumor growth. Inhibition of the CXCR2 receptor attenuates melanoma cell proliferation[45], whereas overexpression of CXCL1, CXCL2, CXCL3 enhances tumor cell colony-forming activity and tumorigenicity in nude mice[46,47]. Macrophage pro-inflammatory chemokine 3α (CCL20), is a CC chemokine where the two cysteine (C) residues lie adjacent to each other, is overexpressed in pancreatic carcinoma cells and infiltrating macrophages adjacent to tumor cells, and enhances migration of TAMs[48]. Angiogenesis is associated with chronic inflammatory states including arthritis, infections, tumor growth and metastasis. The Glutamic acid-Leucine-Arginine (ELR) motif upstream of the CXC domain in the chemokine family can stimulate endothelial cell chemotaxis and retains pro-angiogenic function. Metastatic potential also appears in part to be governed by chemokine receptors and chemokine signaling. CXCL12 [stromal-cell derived factor-1 (SDF-1)] is a product of resting cells in multiple organs and also myofibroblasts. Binding to its receptor CXCR4 appears to play a critical role in cancer metastasis. Use of CXCR4 antibodies can limit CXCL12-mediated chemotaxis of mammary cells to distant organs during metastasis[49]. Indeed the amount of CXCR4 expression in primary human tumors correlates with the extent to which metastasis to the lymph nodes occurs in colorectal, breast, liver and esophageal cancer[50-52]. The critical threshold of pro-inflammatory cytokines reached by the myriad of contributing cells within the local tumor environment may augment the invasive capacity of transforming cells in a para- and autocrine fashion. For example, autocrine TNF-α upregulates the expression of CXCR4 in ovarian cancer[53].

### IAP

Recently, the inhibitor of apoptosis (IAP) family of proteins have been shown to play a significant role in cancer-related inflammation and metastasis[54]. Alterations in IAPs are found in many types of human cancer including HCC and are associated with chemoresistance, disease progression, and poor prognosis[55]. IAPs function by regulating caspases (cysteine proteases that are involved in apoptosis) and through ubiquitin (Ub)-dependent activation of NF-κB transcription factors. IAP1 (cIAP1; encoded by *BIRC2*), cIAP2 (encoded by *BIRC3*) and XIAP (encoded by *BIRC4*) have particular roles that have been demonstrated in tumor maintenance and progression and deserve particular attention. Using a model of breast cancer metastasis with MDA-MB-231 cells and also MCF-7 cells stably transfected with survivin, Mehrotra and colleagues demonstrated that the

survivin-XIAP complex activates NF-κB and increases metastasis in a splenic injection model of hepatic metastasis[56]. Amplification of chromosome 11q22 containing the *BIRC2* and *BIRC3* exons, occurs at high frequency in hepatocellular carcinoma with cIAP1 and cIAP2 functioning as key mediators of TNF-α-induced activation of NF-κB[55]. TNF-related apoptosis-inducing ligand (TRAIL) induces apoptosis in hepatocellular cancer in a cIAP1-dependent fashion[54].

### *Other signals*

Hepatic EMT defined as loss of cell polarity and decrease in cell-cell adhesion was shown to be associated with reduced hepatocyte nuclear factor (HNF)-4 expression while restoration of HNF-4α1 expression in aggressive HCC cells re-programmed the cells into resting hepatocytes[57]. Analysis of Wnt/β-catenin signaling demonstrated that nuclear accumulation of β-catenin results in loss of epithelial markers and increases expression of hepatic mesenchymal markers such as M2-pyruvate kinase (M2-PK) and cytokeratin (CK19)[58]. *In vivo* loss of E-cadherin, upregulation of Twist (a negative inhibitor of E-cadherin transcription) and reduction of cytosolic β-catenin correlate with more invasive HCC phenotypes, increases metastasis and reduces patient survival[59-61]. Recent studies have also shown that HCV core proteins lower Smad3 expression and decrease E-cadherin expression promoting EMT in human HCC cells[62], while hepatitis B virus encoded HBX upregulates STAT5b in HCC cell lines to augment EMT and cell invasion by repressing E-cadherin[63].

## CONCLUSION

Chronic inflammation and fibrosis in the liver can generate a local environment or niche that supports oncogenesis, tumor progression and metastasis, and in this regard the generation of hepatocellular carcinoma parallels the key inflammatory physiology that is observed in wound healing and EMT. The current increased interest in cancer-associated inflammation may identify targets that modulate the more advanced or metastatic phenotypes in cancer and may yield novel therapeutic options.

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BRIEF ARTICLE

# LOH-profiling by SNP-mapping in a case of multifocal head and neck cancer

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## Abstract

**AIM:** To introduce an approach for the detection of putative genetic host factors that predispose patients to develop head and neck squamous cell carcinomas (HNSCC).

**METHODS:** HNSCC most often result from the accumulation of somatic gene alterations found in tumor cells. A cancer-predisposing genetic background must be expected in individuals who develop multiple cancers, starting at an unexpectedly young age or with little carcinogen exposure. Genome-wide loss of heterozygosity (LOH) profiling by single nucleotide polymorphism microarray mapping was performed in a patient with a remarkable history of multifocal HNSCC.

**RESULTS:** Regions of genomic deletions in germline DNA were identified on several chromosomes with a remarkable size between 1.6 Mb and 8.1 Mb (mega base-pair). No LOH was detected at the genomic location of the tumor suppressor gene *P53*.

**CONCLUSION:** Specific patterns of germline DNA deletions may be responsible for susceptibility to HNSCC and should be further analyzed.



**Key words:** Genome-wide analysis; Head and neck cancer; Loss of heterozygosity; Multifocal cancer; Single nucleotide polymorphism microarray; Squamous cell carcinoma

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## INTRODUCTION

Head and neck squamous cell carcinomas (HNSCC) account for approximately 5% of all carcinomas in industrialized countries and represent the sixth most common human neoplasm with an estimated annual worldwide incidence of 500 000 new cases. It is primarily a disease of older age, occurring most frequently in the 6th and 7th decades of life[1]. Although significant advances in radiation therapy, chemotherapy and surgical techniques have improved organ preservation and the overall quality-of-life of patients with HNSCC, the long-term survival rate for this disease has not improved significantly during the past 20 years and remains unchanged at approximately 50%[2,3]. Tobacco-and alcohol consumption are highly significant etiological risk factors associated with the development of HNSCC, with more than 90% of these tumors occurring in individuals who smoke or drink[4]. However, beside these established risk factors individual variations in genetic susceptibility must contribute sig-

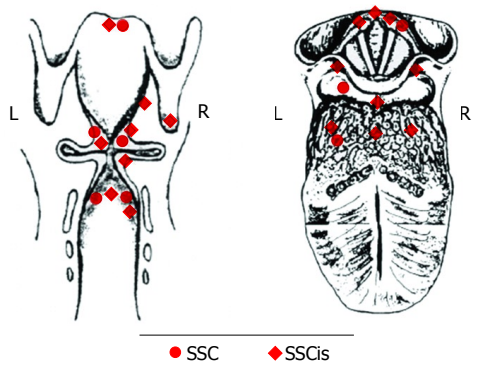


**Figure 1 Overview of the locations with biopsy-proven squamous cell carcinoma and squamous cell carcinoma *in situ* in the upper aerodigestive tract of the patient (profile of the hypopharynx, larynx and trachea and top view of the tongue, hypopharynx and larynx).** L: Left; R: Right; SSC: Squamous cell carcinoma; SSCis: Squamous cell carcinoma *in situ*.

nificantly to the development of HNSCC, because some patients develop this cancer in the absence of identifiable life-style or environmental factors, at an exceptionally young age or at multiple locations. About 5% of patients with HNSCC develop synchronous or metachronous second primary cancers of the aerodigestivetract[5]. The concept of "field cancerization" hypothesizes that regions of the mucosal epithelium, although normal in appearance, are "preconditioned" by exposure to carcinogenic agents, thus priming them for the subsequent development of invasive lesions[3]. While the development of most human malignancies is caused by the accumulation of somatic gene alterations in the tumor cell, a genetic host factor for the development of HNSCC in the form of an additional predisposition on the genomic level can be supposed in patients with multiple primary head and neck tumors[4]. Loss of heterozygosity (LOH) is an indicator of genomic instability and has traditionally been characterized by the additional mutation of an intact allele at chromosomal locations of a pre-existing allelic heterozygosity, that is the somatic conversion of heterozygous germline alleles to homozygosity[1]. Instead of analyzing somatic LOH in DNA isolated from tumor tissue, the intention of this report is to introduce an approach for the detection of potential host factors that predispose patients to develop HNSCC. Therefore, the remarkable results of genome-wide LOH profiling in a patient with multifocal head and neck cancer are presented.

## MATERIALS AND METHODS

The study was performed in accordance with the guidelines of the Helsinki Declaration of 1975, as revised in 1983 and the local institutional review board approved this project. In 2000, a 49-year-old man was admitted to our department for further management of multifocal invasive and microinvasive SCC as well as multifocal carcinoma *in situ* (SCCis) of the upper aerodigestive tract. He had stopped smoking cigarettes after 15 pack-years in 1988 at the age of 37. He had no history of alcohol abuse. In 1997, at the age of 46, excision biopsy of an enlarged cervical lymph node had revealed a metastasis of a poorly-differentiated SCC of unknown primary. Despite radiological investigations, panendoscopy with directed biopsies of Waldeyer's ring, and bilateral tonsillectomy, a primary tumor site was not detected at that time. After 3 cycles of chemotherapy with cisplatin, 5-fluorouracil and folinic acid, radiation therapy was administered to the neck with a cumulative radiation dose of 50 Gy. As the primary tumor site remained unclear and at the request of the patient, the larynx and the pharynx were not irradiated. Between July 1998 and March 2007 control investigations including panendoscopy performed at regular 3 to 6 mo intervals revealed multifocal invasive or microinvasive SCC and SCCis. An overview of their locations is presented in Figure 1. The patient was repeatedly treated by local tumor resection, redifferentiation therapy with interferon alpha, isotretinoin and alpha-tocopherol as well as photodynamic therapy requiring temporary tracheostomy. Long-term improvement has not been attained up to now. No distant metastases have been found. Genome-wide LOH profiling by high-density single nucleotide polymorphism (SNP) microarray mapping was performed from peripheral blood using the Affymetrix 50K XbaI SNP Mapping Array. Genomic DNA was extracted from lymphocyte serum samples using the QIAGEN Blood and Cell Culture DNA kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). The Affymetrix 50K Xba I SNP mapping array includes around 58 000 SNPs. The chips and reagents were obtained from Affymetrix and the assays were carried out according to the manufacturer's instructions. Briefly, 250 ng of genomic DNA were digested with *Xba*I and then ligated to adapters. A generic primer that recognizes the adapter sequence was used to amplify adapter-ligated DNA fragments with polymerase chain reaction (PCR) conditions optimized to preferentially amplify fragments in the 250-2000 bp size range in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, United States). After purification with the QIAquick purification system (Qiagen), a total of 40 μg of PCR product was fragmented and a sample was visualized on 4% TBE agarose gel to confirm the right average size. The fragmented DNA was then labeled with biotin and hybridized to the GeneChip Mapping 50K Xba I Set for 20 h. The arrays were washed and stained using the Affymetrix fluidics Station 450 and scanned with the GeneChip Scanner 3000 G7 (Affymetrix, Santa Clara, CA, United States). The GeneChip Genotyping analysis software was used to analyze feature intensity data stored in the GCOS Database, and provided high-throughput and accurate genotyping analysis.

## RESULTS

Results are summarized in Figure 2 and Table 1. Regions of genomic deletions were identified on chromosomes 3,

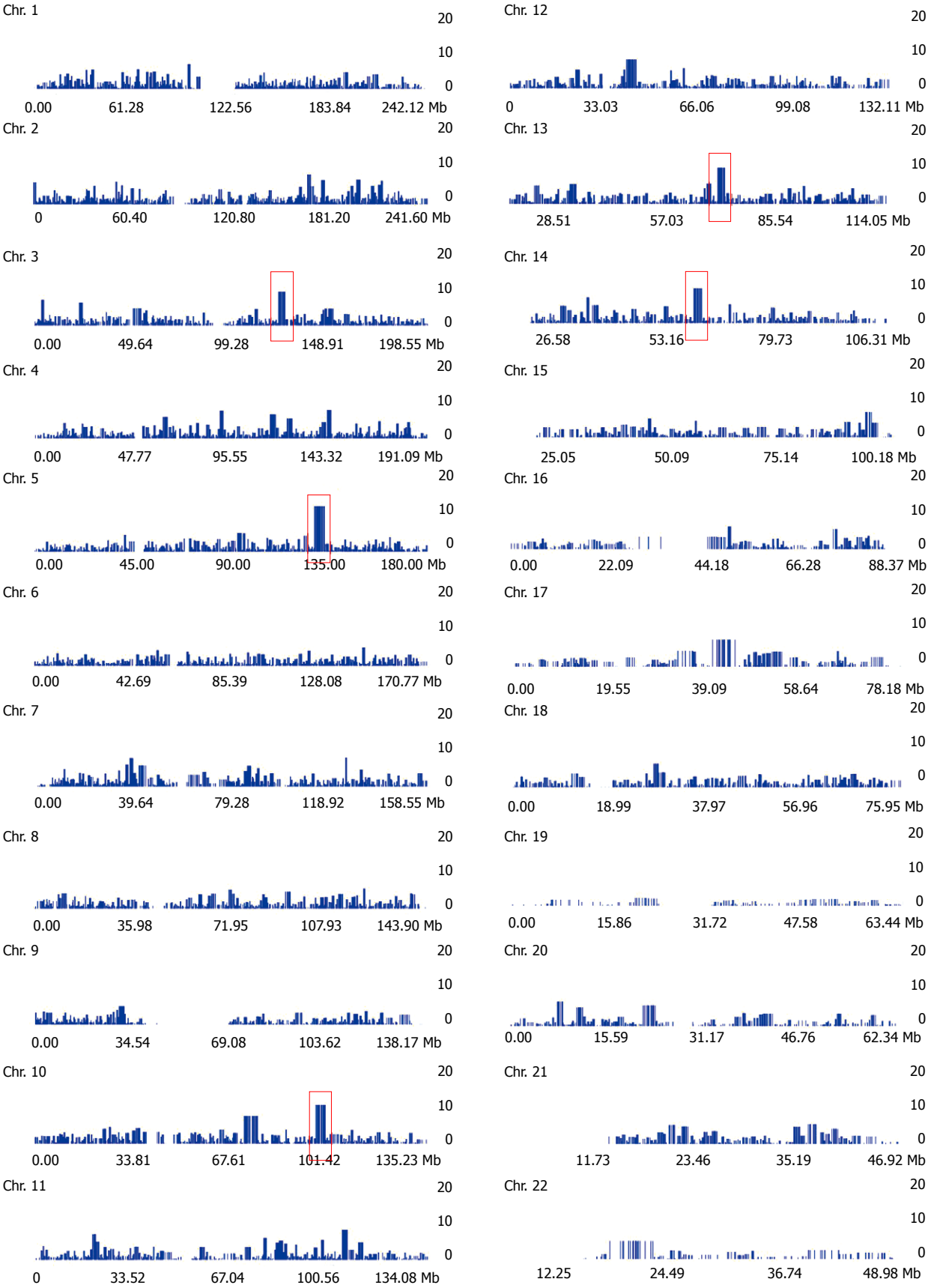


**Figure 2 Single nucleotide polymorphism 50K XbaI genome-wide loss of heterozygosity profiling using the AffymetrixGeneChip Assay System.** Regions of genomic deletions between 1.6 Mb and 8.1 Mb in size were identified on 5 chromosomes (red labeling). The x-axis shows physical position along the respective chromosome. The y-axis indicates -log10P values where homozygous stretches are due to loss of heterozygosity.

**Table 1 Summary of loss of heterozygosity regions on five chromosomes**

| Chromosome | Genomic location | Size |
|---|---|---|
| Chromosome 3 | 123.2-126.4 Mb | 3.2 Mb |
| Chromosome 5 | 128.3-132.8 Mb | 4.5 Mb |
| Chromosome 10 | 97.1-105.2 Mb | 8.1 Mb |
| Chromosome 13 | 71.3-72.9 Mb | 1.6 Mb |
| Chromosome 14 | 59.2-61 Mb | 1.8 Mb |

5, 10, 13 and 14 ranging in size from 1.6 to 8.1 Mb (mega base-pair). It can be hypothesized that the identified regions of genomic deletions harbor candidate genes that might be associated with cancer susceptibility or a function in tumor suppression. No LOH was detected at the genomic location of the tumor suppression gene *P53*, which is located on chromosome 17 (17p13.1).

## DISCUSSION

Most human cancers are characterized by genetic instabilities. Cancer arises when gene mutations lead to an abnormal cell resulting in clonal proliferation, aggressive spread, or prevention of apoptosis. As with other solid tumors, HNSCC is believed to originate *via* a multistep process which involves defects in proto-oncogenes, suppressor genes, and several other functionally essential genes. Mathematical models estimate that approximately seven to ten individual genetic alterations must accumulate in the epithelia of the upper aerodigestive tract for the development of cancer[4,6-8]. The vast majority of these mutations occur after birth and are found only in the cancer cells themselves. Inactivation of the *P53* tumor suppressor gene is the most common genetic alteration in all cancer types and is extremely common in squamous cell carcinoma, with approximately 50% of the lesions expressing a mutant form of the protein[4]. Most studies on genetic instability in HNSCC analyze somatic mutations in the cancer cell themselves using DNA from microdissected tumor tissue[9,10]. LOH is a frequent mechanism of inactivation of tumor suppressor genes where one allele is already altered. LOH profiling is a powerful molecular genetic approach for high-resolution screening of genomic alterations. The Affymetrix 50K SNP mapping array provides a high throughput tool for genotyping and genome-wide LOH profiling[9-12]. One advantage of the use of DNA microarray technology is the ability to screen the entire genome with small amounts of DNA[13]. The most common method is to directly compare genotypes between tumor and paired germline DNA, in which only SNPs that are heterozygous in the germline are informative as to whether LOH has taken place[11]. About 5% of cancers are thought to arise in the context of a cancer-preconditioning genetic background which predisposes for the development of more than one tumor[5]. A cancer-predisposing genetic background can be expected in individuals who develop multiple cancers during their lifetime, starting at an unexpectedly young age or develop HNSCC with little carcinogen exposure, e.g., smoking and drinking. We present here a novel approach to identify predisposing host factors for the development of HNSCC. Genome-wide LOH profiling by high-density SNP microarray mapping at the genomic level in a patient with an exceptional history of multifocal head and neck cancer localized a number of genomic deletions in the germline DNA with remarkable sizes. The advantage of SNP mapping arrays is that they provide marker densities that enable the identification of "LOH regions" without the use of paired "normal" DNA. Statistical software analysis are applied to identify strings of consecutive homozygous SNPs that are longer than would be expected to appear by chance alone and, as such, every SNP is informative[10]. Frequent allelic loss at specific loci in the germline DNA of patients with multiple primary HNSCC may therefore indicate the location of putative genes, whose malfunction can be evaluated as a host factor predisposing the patient for the development of invasive carcinomas. Based on our observation we hypothesize that patterns of germline deletions at multiple sites may favor the accumulation of genetic defects in the epithelia of the aerodigestive tract and finally in the cancer cell and predispose for the development of HNSCC. To validate the replication of our findings with an increased number of patients, this observation should be systematically studied using the presented approach in a selected group of patients who clinically appear particular susceptible for the development of HNSCC.

## COMMENTS

### Background

Head and neck squamous cell carcinomas (HNSCC) account for approximately 5% of all carcinomas in industrialized countries and are primarily a disease of older age. More than 90% of HNSCC occur after tobacco or alcohol abuse. However, individual variations in genetic susceptibility must contribute significantly to the development of HNSCC because some patients develop this disease at an exceptionally young age, without the established risk factors of alcohol and tobacco consumption or at multiple primary sites in the head and neck.

### Research frontiers

Loss of heterozygosity (LOH) is an indicator of genomic instability and has traditionally been analyzed at the somatic level in DNA isolated from tumor tissue. The authors analyzed LOH at the genomic level in a patient with multiple primary HNSCC to identify genetic host factors that predisposed the patient to develop this disease.

### Innovations and breakthroughs

The authors present a novel approach to identify predisposing host factors for the development of HNSCC in patients that clinically appear susceptible to this disease. They localized a number of genomic deletions with remarkable sizes in the germline DNA of a patient with an exceptional history of multifocal HNSCC by genome-wide LOH profiling.

### Applications

The study results suggest that this approach should be systematically applied and the observation studied in a larger group of patients who clinically appear predisposed for the development of HNSCC. This approach might help to identify patients susceptible to head and neck cancer particularly in the follow-up of this disease.

### Terminology

Single nucleotide polymorphisms (SNPs) are short polymorphism in the human DNA. For each SNP the genomic location and frequency distribution in the aver-

age human population is known. The statistical analysis of the hybridization pattern on SNP arrays can indicate LOH regions with high probability. The technique was used for genome-wide LOH profiling to identify regions of genomic deletions.

### *Peer review*

The authors present an interesting approach and an interesting finding in a single case study. Future research should try to validate the findings with an increased number of patients.

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CASE REPORT

# Colon cancer in a patient with underlying aplastic anemia: A clinical challenge

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## Abstract

The association of gastrointestinal malignancy with aplastic anemia has rarely been reported in the literature. Although it is not clear whether there is any direct relationship between aplastic anemia and gastrointestinal cancers, a retrospective analysis did suggest the notion that patients with aplastic anemia might have a higher incidence of colorectal cancer. Here, we report the diagnostic and therapeutic challenges in managing a patient with aplastic anemia and advanced colorectal cancer. Early diagnosis is challenging due to overlapping symptomatology and clinical features, increased risk of diagnostic procedures, and confounding complications arising from aplastic anemia and its treatment. A high index of suspicion and multidisciplinary input are essential.



**Key words:** Aplastic anemia; Colon cancer; Gastrointestinal malignancy

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## INTRODUCTION

The association of aplastic anemia with gastrointestinal malignancy has rarely been described in the literature, except in patients with Fanconi's anemia. In fact, Fanconi's anemia is a rare autosomal recessive disorder, consisting of aplastic anemia and genetic predisposition to cancers. In this congenital condition, the susceptibility to the development of cancer is accounted by the inherited chromosomal fragility and genetic defects in DNA repair mechanisms[1]. Although it is not clear whether there is any direct relationship between aplastic anemia and solid tumors, a retrospective analysis of 734 patients suggested that aplastic anemia patients did show a higher incidence of colorectal cancer (CRC)[2]. There is also evidence that solid cancers occur after treatment of aplastic anemia[3,4]. On the other hand, there has been interesting speculation that autoimmunity against hematopoietic stem cells which mediates acquired aplastic anemia, may actually be anti-neoplastic[5].

Here, we report the diagnostic and therapeutic challenges in managing a patient with aplastic anemia who subsequently developed advanced CRC.

## CASE REPORT

A 55-year-old photographer initially presented with the incidental finding of pancytopenia. He had otherwise good past health. The patient had prior workplace ex-

posure to chemicals used in film development. Physical examination was unremarkable. White cell count (WCC) was 3.8 × $10^9$/L (normal: 4 × $10^9$/L-11 × $10^9$/L) with a normal differential count, hemoglobin 89 g/L (normal: 135-175 g/L) and platelet count 40 × $10^9$/L (normal: 150 × $10^9$/L -400 × $10^9$/L). Anemic workup, particularly vitamin B12 and folate levels, were normal. Reticulocyte count was 1%. Both Ham's test and urine hemosiderin were negative. Finally, bone marrow examination showed hypocellular and hypoplastic marrow without increased blasts, dysplastic features or other abnormal cellular infiltration. Thus, the diagnosis of acquired aplastic anemia was made. The patient was started on cyclosporine 50 mg twice daily thereafter with stable blood counts, until two years later, when he was found to have reducedhemoglobulin level to 5.1g/dL necessitating repeated red cell transfusions. At that time, the total WCC was 2.7 × $10^9$/L with neutropenia of 1.3 × $10^9$/L, platelet count of 11 × $10^9$, and reticulocyte count less than 1%. Repeated bone marrow examination showed particularly severe erythroid and megakaryocytic hypoplasia, while excluding hypoplastic leukemia andmyelodysplastic syndrome. He was thus diagnosed as having severe aplastic anemia (SAA) and was treated with a course of intravenous horse anti-thymocyte globulin at a dose of 2720 mg daily (40 mg/kg per day) for 4 d. He subsequently developed severe gastrointestinal bleeding. Colonoscopy was not performed at that juncture due to low platelet count. Moreover, the patient had repeated episodes of septicemia respectively caused by *Escherichia coli, Enterococcus gallinarium* and cytomegalovirus, which were treated with prolonged courses of broad-spectrum antibiotics including ceftazidime, ertapenem and meropenem, and foscarnet. Plain computed tomography (CT) of the abdomen and gallium scintigraphy were arranged to identify possible occult sources of recurrent bacteremia. A contrast CT scan was not performed due to impaired renal function. The plain CT images revealed four hypodense lesions in the liver. Gallium scintigraphy showed no corresponding uptake in the liver lesions but increased uptake at the proximal descending colon instead. Subsequent colonoscopy showed a fungating tumor at the proximal descending colon with biopsy confirmed adenocarcinoma. Positron emission tomography (PET) demonstrated a primary colon cancer with multiple liver metastases (Figure 1). Carcinoembryonic antigen was also grossly raised (460 ng/mL). He was referred to the medical oncologist for consideration of systemic therapy for metastatic CRC. Systemic chemotherapy was not recommended due to his underlying aplastic anemia and cytopenia. Single agent cetuximab was suggested but he could not afford the treatment. He was thus treated with best supportive care only and died of advanced CRC approximately 8 mo after the diagnosis.

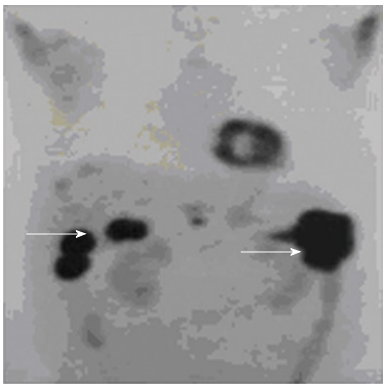

**Figure 1 Positron emission tomography-computed tomography showing the hypermetabolic primary colon cancer (right arrow) and multiple liver metastases (left arrow).**

## DISCUSSION

Despite the speculated association between aplastic anemia and gastrointestinal malignancy as summarized in the Introduction, we cannot ascertain such a causal relationship between aplastic anemia and CRC in our patient. In fact, the co-existence of these two diseases may be coincidental as CRC is a commonly encountered malignancy nowadays with a rising incidence in most countries. In the presented patient, there could have been occupational exposure to benzene and its metabolite hydroquinone, which are present in radiographic developer solutions, and have been reported to be both toxic to the bone marrow and possibly carcinogenic[6,7]. Nonetheless, a few interesting diagnostic and therapeutic challenges in managing this case are worth highlighting.

First, colon cancer and aplastic anemia share many similar presenting symptoms and clinical features, leading to difficulties in the diagnosis of one condition when the other is present. Gastrointestinal bleeding is a well-known complication and presenting symptom in colorectal cancer[8], and also, albeit less commonly, in aplastic anemia. A recent study showed that up to 12% of patients with SAA developed overt gastrointestinal bleeding, especially lower gastrointestinal bleeding, due to neutropenicenterocolitis or solitary ulcer[9]. Chronic thrombocytopenia frequently present in bone marrow failure states, including aplastic anemia, can also be associated with active gastrointestinal bleeding[10]. Anemia is another common clinical feature encountered in both diseases. Notably, patients with colorectal cancer can present with anemia alone, related to iron deficiency and chronic blood loss[8], while anemia is disease-defining in aplastic anemia. Moreover, recurrent infections may occur in both CRC and aplastic anemia. *Streptococusbovis* septicemia is classically associated with colorectal cancer[11]; other organisms include *Enterobacter*[12] and *Clostridium* species[13]. Infections that are seen in aplastic anemia also include *Streptococcus* and *Clostridium* species, in addition to *Escherichia coli* and others[14]. In the present case where an initial diagnosis of aplastic anemia had been established, new symptoms of gastrointestinal bleeding, worsening anemia and recurrent bacterial septicemia were thought to be related to the complications or pro-

gression of aplastic anemia, thus delaying investigations in search of other important causes including colorectal malignancy.

Second, there is a higher risk of performing invasive diagnostic procedures in patients with aplastic anemia. Neutropenia and thrombocytopenia increase rates of infection and bleeding with colonoscopy and biopsy. Due to abnormal renal function caused by a combination of sepsis and administration of renal-toxic drugs including cyclosporine and foscarnet, contrast-enhanced CT could not be performed to delineate the underlying pathology in order to avoid contrast nephropathy.At the time of reporting, the PET imaging modality was not readily accessible especially as the patient was financially challenged, although this could potentially serve as a less invasive diagnostic means in patients with limiting co-morbidities suspected of colon cancer.

Last but not least, the management of either aplastic anemia or colon cancer is highly dependent on the treatment response, toxicities and complications of the other condition among other factors, often presenting a clinical dilemma. In view of poor marrow tolerance with low blood counts and recurrent septicemia caused by prolonged neutropenia and the administration of immunosuppressive agents, systemic chemotherapy as treatment of metastatic CRC was decided against in the present case as there was a high probability of treatment-related morbidity and mortality. Unfortunately, the patient could not afford targeted therapy as he might potentially have benefited from it without a significant drop in blood counts.

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## Events Calendar 2012

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Biomarkers Summit Egypt
London, United Kingdom

January 25-26, 2012
Multi-Disciplinary Appoaches to Cancer Therapy
Dubai, United Arab Emirates

January 26-27, 2012
3rd National Conference: Renal and Bladder Cancer 2012
London, United Kingdom

January 30-31, 2012
2nd Annual Clinical Trials in Oncology
Rome, Italy

February 2-3, 2012
Stem Cells 2012 Conference and Exhibition
San Diego, CA, United States

February 6-8, 2012
Mahidol International Conference on Infections and Cancers 2012
Bangkok, Thailand

February 12-17, 2012
Keystone Symposia: Cancer and Metabolism
Alberta, Canada

February 22-25, 2012
Excellence in Oncology
Istanbul, Turkey

March 8-10, 2012
10th International Congress on Targeted Anticancer Therapies
Amsterdam, Netherlands

March 9-10, 2012
13th European Congress: Perspectives in Lung Cancer
Amsterdam, Netherlands

March 14-16, 2012
BTOC-11 Biological Therapy of Cancer
Munich, Germany

March 15-17, 2012
3rd Conference on Therapeutic Resistance in Cancer
Quebec, Canada

March 29-30, 2012
Modern methods of diagnosis and treatment of malignant tumors
Kiev, Ukraine

April 13-15, 2012
Asian Oncology Summit 2012
Singapore, Singapore

April 20-21, 2012
Diagnosis and treatment of advanced forms of prostate cancer, bladder cancer and kidney cancer
Kiev, Ukraine

April 20-22, 2012
The 9th Meeting of Asian Society for Neuro-Oncology
Taipei, Taiwan

April 26-28, 2012
3rd International Video Workshop on Radical Surgery in Gynaecological Oncology
Prague, Czech Republic

April 28, 2012
Issues in Pediatric Oncology
Kiev, Ukraine

May 5-6, 2012
Radiation Research Methods as A Diagnostic and Therapeutic Support in Oncology
Kiev, Ukraine

May 17-18, 2012
Eurasian forum on the management of patients with tumors of the gastrointestinal tract
Uman, Ukraine

June 16-17, 2012
Issues of Neurosurgery, vascular neurosurgery, neurooncology, spinal surgery and spinal cord
Kiev, Ukraine

July 7-10, 2012
22nd Biennial Congress of the European Association for Cancer Research
Barcelona, Spain

July 21-28, 2012
Cancer In Women
Hawaii, HI, United States

July 25-27, 2012
5th Latin American Conference on Lung Cancer
Rio de Janeiro, Brazil

August 27-30, 2012
UICC World Cancer Congress 2012
Québec, Canada

September 6-8, 2012
The 8th International Jordanian Oncology Society Conference
Amman, Jordan

September 27-28, 2012
Current issues of diagnosis and treatment of oncogynecology diseases
Ivano Frankivsk, Ukraine

September 27-29, 2012
European Conference of Oncology Pharmacy
Budapest, Hungary

October 5-8, 2012
44th Congress of the International Society of Paediatric Oncology
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October 13-16, 2012
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October 23-26, 2012
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October 27-28, 2012
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November 6-9, 2012
24th EORTC-NCI-AACR Symposium on "Molecular Targets and Cancer Therapeutics"
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World Journal of Clinical Oncology

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### *Acknowledgments*

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### *Format*

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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

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Write as mean ± SD or mean ± SE.

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

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

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***Italics***

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
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