Obesity, Inflammatory Signaling, and Hepatocellular Carcinoma—An Enlarging Link

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There is growing evidence that obesity increases the risk of hepatocellular carcinoma (HCC). In a recent issue of *Cell*, Park et al. show that IL-6 and TNF signaling through activation of STAT3 are critical for obesity-promoted HCC development, underscoring the important role of inflammatory pathways in hepatocarcinogenesis.

Obesity represents an important public health problem with an explosive increase worldwide; at present, approximately 300 million people are obese. Obesity elevates the risk of cancer of all types including hepatocellular carcinoma (HCC). In a recent large study from the United States, the relative risk of death from HCC in obese patients with body mass index (BMI) $\geq$ 35 kg/m^2 was 4.52 and 1.68 times higher among men and women, respectively, compared with their reference groups (Calle et al., 2003). Considering the current epidemiological trends, the incidence of HCC is likely to increase precipitously, particularly in those obese patients who develop nonalcoholic steatohepatitis (NASH), hepatic fibrosis, and cirrhosis.

Molecular links between inflammation and liver cancer have progressively emerged, and a number of studies have uncovered several mechanisms, including the recently reported role of lymphotoxin signaling in HCC development (Villanueva et al., 2009). Nonetheless, there is still only elemental insight into the specific pathogenic mechanisms linking obesity and liver cancer. Accumulation of intracellular lipids increases the demand on the endoplasmic reticulum (ER), which integrates many metabolic processes. ER dysfunction leads to production of reactive oxygen species (ROS), provoking oxidative stress and activation of inflammatory pathways (e.g., NF-κB and JNK signaling) (Zhang and Kaufman, 2008). Oxidative stress can also induce DNA damage that leads to genomic instability. Moreover, the adipose tissue is increasingly viewed not simply as a reservoir of stored energy, but rather as an active secretory organ, which releases inflammatory cytokines and hormones (e.g., IL-1, TNF, adiponectin, leptin). In parallel, there is consistent epidemiological evidence associating high circulating IGF-I levels with the risk of breast, colon, and lung cancers, but the roles of insulin resistance and IGF signaling in the initiation of HCC are not clearly understood. Once overt HCC has developed, however, dysregulation of IGF signaling driven by IGF2 occur in a subgroup of patients, resulting in downstream activation of ERK and Akt pathways.

In a recent issue of *Cell*, Park et al. demonstrate that interleukin 6 (IL-6) and tumor necrosis factor (TNF) are required for the development of experimental obesity-induced HCC (Park et al., 2010). The authors propose a novel pathogenic mechanism linking obesity and liver cancer. Lipid accumulation in obesity induces a low-grade inflammatory response, which in turn increases IL-6 and TNF expression by adipose tissue and Kupffer cells resulting from hepatocyte death. The authors demonstrate that these two molecules are critical for the development of steatohepatitis and the induction of cell proliferation through JAK/STAT and ERK activation leading to HCC development. They further argued that once a genomic hit initiates the process of carcinogenesis, obesity acts as a bona fide liver tumor promoter through these molecular mechanisms (Figure 1).

Mice were pretreated with the hepatic procarcinogen diethylnitrosamine (DEN) to induce liver tumors and then placed on high-fat diet (HFD) or low-fat diet (LFD). Those on HFD developed larger liver tumors with increased incidence than tumors arising in mice fed a LFD. Similar results were obtained with genetically obese mice (*Lep*^{ob}), compared with the corresponding wild-type animals. Lower apoptotic rates and an increased number of proliferating cells in HCC arose in obese mice compared with HCCs in lean mice, suggesting that obesity can stimulate compensatory proliferation of hepatocytes. Molecular analysis uncovered phosphorylation of STAT3, JNK, ERK, and S6, and higher expression of TNF and IL-1β in HCCs from obese mice, associated with increased circulating levels of IL-6.

The role of obesity in tumor progression was further reinforced by the fact that HCCs originating from transplanted hepatoma cells grew more quickly in mice on a HFD compared with those on a LFD. The tumor-promoting activity of IL-6 and TNF was confirmed by demonstrating attenuation of tumorigenesis in mice lacking either IL-6 or TNFR1. Importantly, absence of either IL-6 or TNFR1 in tumor-bearing mice reduced lipid accumulation and infiltration of both macrophages and neutrophils induced by HFD.

In aggregate, these findings indicate that obesity promotes chronic hepatic inflammation and fosters a permissive environment for the proliferation and malignant transformation of hepatocytes. IL-6 activates the JAK/STAT pathway through phosphorylation and translocation of STAT3 into the nucleus, where it

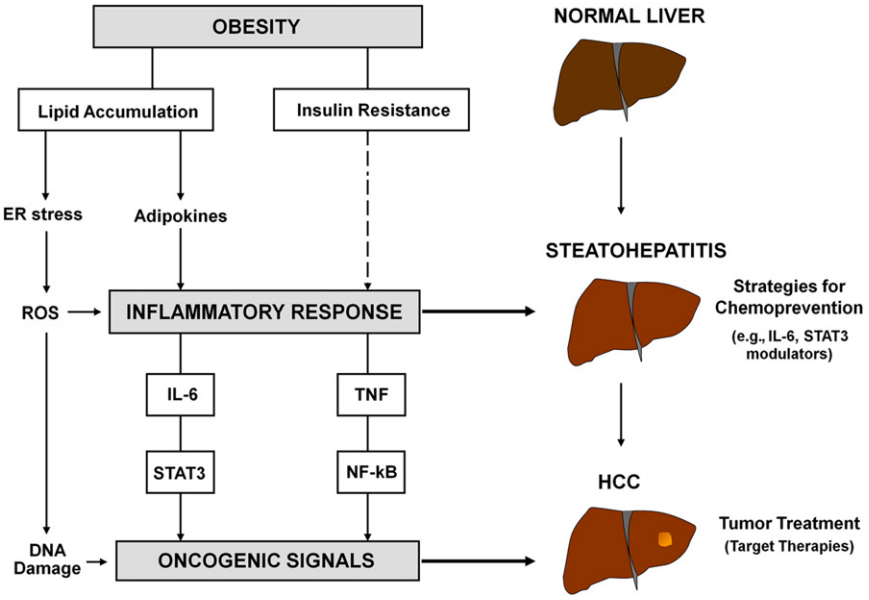

Figure 1. Principal Events Involved in Obesity-Promoted Hepatocellular Carcinoma

In obese patients, accumulation of lipids in the liver and insulin resistance promote activation of an inflammatory response. Adipocites produce a variety of cytokines, collectively named adipokines (e.g., adiponectin, leptin, and IL-1), which contribute to activation of inflammatory pathways. At the same time, lipid accumulation increases demand on the endoplasmic reticulum (ER), leading to uncontrolled production of reactive oxygen species (ROS). ROS stimulate inflammatory signaling and induce oxidative damage, including strand breaks and nucleotide modifications. In a recent issue of *Cell*, Park et al. demonstrate that obesity induces production of IL-6 and TNF cytokines, which are required for the initiation and progression of hepatocellular carcinoma (HCC). IL-6 ad TNF activate STAT3 and NF-κB, respectively, which promotes cell proliferation of damaged cells through transcriptional activation of genes involved in cell growth and survival. Accumulation of molecular alterations and pathway activation in damaged hepatocytes contribute to HCC development.

induces the expression of genes involved in cell-cycle progression and suppression of apoptosis. Concentrations of IL-6 in serum are increased in states of chronic liver inflammation, including alcoholic hepatitis, and chronic hepatitis B and C, infections that predispose to HCC. Moreover, IL-6 signaling promotes chemically induced hepatocellular carcinoma, and gender-related differences in its expression in genetic mouse models might partially explain the 3:1 male/female ratio of HCC observed in clinical practice (Naugler et al., 2007). Similarly, TNF may also be tumorigenic by promoting cell survival through the induction of genes encoding NF-κB-dependent antiapoptotic molecules and by stimulating the production of ROS. As a result, the NF-κB pathway is recognized as a critical promoter of inflammation-linked cancers, including liver tumors.

From a translational perspective, the study by Park et al. recapitulates most of the key events underlying HCC development in obesity-related fatty-liver disease in humans (Figure 1). Specifically, the hepatic lipid accumulation and inflammation of obese mice resemble the pathological features of steatohepatitis commonly observed in obese patients. Moreover, IL-6 and TNF signaling have also been implicated in human HCC. Among them, the JAK/STAT pathway, which is induced by IL-6, is ubiquitously activated in HCC compared with nontumoral livers, mainly through inactivation of Suppressor of Cytokine Signaling (SOCS) genes by promoter methylation (Calvisi et al., 2006). In a recent large human study, genes downstream of IL-6 signaling were enriched within a signature portending poorer survival and de novo recurrence after HCC resection (Hoshida et al., 2008). Interestingly, this signature was generated from the tissue surrounding the HCCs, implicating a permissive effect of enhanced IL-6 that accelerates the emergence of a tumor. Thus, the extent of liver damage and the presence of a proinflammatory environment can be reflected in a clinically available gene signature that has prognostic implications.

A major feature of most HCCs that is lacking in the model from Park et al. is the presence of advanced fibrosis and cirrhosis. In human disease, these pathologic features greatly augment the risk of HCC (Llovet et al., 2003), although underlying mechanisms are not clearly delineated. Current models suggest that enhanced signaling by platelet-derived growth factor receptor, hedgehog, Toll-like receptor, and PTEN may be implicated in this process. (Trimboli et al., 2009).

Given the relevance of inflammatory signaling to the development and progression of HCC, modulators of cytokines and mediators of pathways involved in their activation represent attractive targets for HCC chemoprevention and therapy (Figure 1). Modulation of the inflammatory response could attenuate chronic exposure to oncogenic signals in the injured liver, thereby preventing the development of HCC. In that regard, selection of populations at high-risk of HCC development are critical to test these types of agents in the preventive scenario. Before initiating a large preventive trial, however, the target population of high-risk obese patients with steatohepatitis should be further refined. Moreover, given that inflammatory cascades are overactivated in established HCC, inhibitors against these pathways (e.g., JAK/STAT, NF-κB inhibitors) could be useful when combined with molecular-targeted agents already approved for the treatment of advanced tumors, such as the multikinase inhibitor sorafenib (Llovet et al., 2008). Although bortezomib—a proteasome inhibitor—has shown discouraging results in early clinical trials, small molecules abrogating JAK/STAT activation can be potential candidates for thorough testing in clinical trials. In conclusion, the study by Park et al. provides a new link between obesity, inflammation, and HCC and reveals IL6, STAT3, and TNF signaling as attractive potential targets for the chemoprevention and treatment of liver cancer.

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DUB-le Trouble for Cell Survival

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Expression of MCL-1 is frequently elevated in cancer and is implicated in the resistance to chemotherapy by the BCL-2 small molecule inhibitor ABT-737. A recent paper in *Nature* identified USP9X as an antagonist of MCL-1 ubiquitinylation and degradation. Often upregulated in tumor cells, USP9X activity influences the response to ABT-737.

The intricate decision processes that dictate cell life and death frequently converge on the BCL-2 family of proteins that control mitochondrial outer membrane integrity and the mitochondrial pathway of apoptosis (Letai, 2008). One family member, antiapoptotic myeloid cell leukemia sequence 1 (*MCL-1*), is an essential survival factor for stem and progenitor cells of multiple cellular lineages, and its overexpression is common in human cancers, including B cell and mantle cell lymphomas, acute lymphoblastic leukemia, chronic myelogenous leukemia, chronic lymphocytic leukemia, and multiple myeloma. High expression of MCL-1 correlates with chemotherapeutic resistance and disease progression, although, unlike BCL-2, chromosomal translocations have not been implicated in dysregulating MCL-1 levels. Instead, cellular signaling regulates MCL-1 function and expression at the posttranslational level and aberrations in signaling lead to elevation of MCL-1 in human cancer. Although posttranslational modifications are known in other BCL-2 family members, MCL-1 is unique in its short half-life, partly because of regulated ubiquitinylation and proteasomal degradation. Cancer cells often violate cellular checkpoints that should induce apoptosis, leading to the hypothesis that cancer cells are "addicted" to antiapoptotic BCL-2 family members that support their survival under adverse conditions. ABT-737, a small-molecule inhibitor of BCL-2, BCL-X_L, and BCL-w, promotes apoptosis in some cancer cells. However, high MCL-1 expression renders cancer cells resistant to ABT-737; thus, MCL-1 expression represents a critical resistance mechanism to ABT-737 efficacy.

Recently, a new player in the control of MCL-1 stability was identified: ubiquitin specific peptidase 9 X-linked (USP9X) (Schwickart et al., 2010). Deubiquitinases (DUBs) are proteins that act to remove conjugated ubiquitin, thereby antagonizing the effect of ubiquitin E3 ligases. RNAi-mediated silencing of USP9X resulted in loss of MCL-1 without affecting its mRNA expression (see Figure 1). Biochemically, USP9X binds to MCL-1 and directly removes degradative Lys-48-linked polyubiquitin chains on the protein. Intriguingly, high levels of MCL-1 correlated with elevated USP9X expression in follicular lymphoma, ductal adenocarcinoma, colon adenocarcinoma, and small-cell lung carcinoma samples. Furthermore, increased expression of USP9X mRNA significantly associated with poor prognosis in a retrospective study of multiple myeloma samples. For determining whether the interaction between USP9X and MCL-1 might affect ABT-737 sensitivity, USP9X was silenced by RNAi in a panel of ABT-737-resistant tumor cell lines. Loss of USP9X expression reduced MCL-1 levels in these cell lines and increased their sensitivity to ABT-737.