—Review—

Development and Maintenance of Cancer Stem Cells under Chronic Inflammation

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Abstract

In many human cancers, tumorigenic potential is not equally shared by all cells but is restricted to phenotypically distinct subpopulations termed cancer stem cells. Cancer stem cells are also capable of both self-renewal and differentiation, and these functional properties have been suggested to play major roles in tumor initiation and progression. The factors responsible for the development of cancer stem cells and their subsequent regulation are unclear, but several chronic inflammatory states have been associated with an increased risk of malignancy. Therefore, it is possible that specific processes associated with chronic inflammation, as well as the adaptation to cellular stress, regulate cancer stem cells. Several factors associated with chronic inflammation, including cytokines, oxidative stress, and hypoxia, induce the activation of specific cellular response programs that can affect the survival, proliferation, metabolism, and differentiation of cancer cells, as well as the self-renewal and quiescence of normal stem cells. In this review, we discuss how these adaptive processes potentially become subverted to enhance the development and function of cancer stem cells.
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Key words: cancer stem cell, chronic inflammation

Introduction

Normal adult tissues are composed of cell of different types which are arranged in a hierarchal manner. Stem cells sit at the top of this hierarchy and give rise to the full range of differentiated cell types capable of specific effector functions. Furthermore, stem cells have the ability to undergo the self-renewal and long-term proliferation that maintain tissue homeostasis and permit regeneration following injury. Accumulating evidence has suggested that some cancers may be similarly arranged in a hierarchical fashion in which self-renewal potential is restricted to functionally and phenotypically unique subpopulations of cancer stem cells (CSCs)[1]. Parallels between the organizations of normal tissues and of malignant tissues were initially described in acute myeloid leukemia (AML). Primary leukemic cells resembling normal hematopoietic stem cells were found to be capable of both producing relatively well differentiated leukemic blasts that phenotypically recapitulated the original clinical specimen and underwent self-renewal, as

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evidenced by serial transplantation in immunodeficient mice[2]. Similar findings have subsequently been described in a wide variety of malignancies[3–7], and these functional attributes have suggested a broad role for CSCs in disease initiation, relapse, and progression[8].

Many chronic inflammatory states have been associated with an increased risk of cancer[9], and several factors arising from chronic inflammation have been implicated in tumor initiation, maintenance, and progression[10]. Some of these factors, including the production of genotoxic reactive oxygen species (ROS) and inflammatory cytokines that support tumor cell proliferation and survival, may act intrinsically to promote tumor formation. Moreover, significant alterations to the extracellular environment, such as increased tissue hypoxia, may also contribute to carcinogenesis. Because many of these processes regulate normal stem cell functions, it is possible that chronic inflammation and the adaptive responses to cellular stress underlie the generation and regulation of CSCs. In this review, we describe how factors arising from chronic inflammatory states might both affect the formation of CSCs and regulate their malignant properties.

Chronic Inflammation and Cancer

The association between inflammation and cancer has been established in a wide variety of diseases. Chronic persistent infections may increase the risk of cancer, and in some diseases, the infectious agent itself may play a direct role in carcinogenesis. For example, cervical carcinoma is associated with human papillomavirus infections, and specific viral genes, such E6 and E7, can transform or immortalize epithelial cells[11]. Human Epstein-Barr virus may also be oncogenic and is associated with a number of human cancers, including Hodgkin and Burkitt lymphomas and nasopharyngeal carcinoma[12]. Although viral gene products may serve as oncogenes, the development of cancer is a rare event compared with infections with each of these viruses. Therefore, other factors, including sustained inflammatory responses, are likely to be required for transformation[13,14].

Other chronic inflammatory states occurring in the absence of infections have also been associated with the development of cancer. Inflammatory bowel diseases, including ulcerative colitis, have been associated with increased rates of colon adenocarcinoma[15,16]. In addition, chronic gastroesophageal reflux disease and Barrett's esophagus may lead to epithelial metaplasia and increase the risk of esophageal carcinoma[17]. Importantly, these sustained inflammatory states have not been definitively associated with a specific infectious agent and are thought to arise from autoimmune or mechanical dysfunction within the digestive system. Therefore, a myriad of factors involved in both tissue injury and repair are likely to influence the development of cancer from these chronic inflammatory states.

Carcinogenic Factors Arising from Chronic Inflammation

Inflammatory Cytokines

Cytokines play several potential roles linking chronic inflammation with the development of cancer. Several cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and transforming growth factor (TGF)-β, are produced during chronic inflammation, and each has been strongly associated with human cancers[10,18]. High production of TNF-α associated with specific polymorphisms has been found to increase the risk of both multiple myeloma and gastric carcinoma and correlates with a poorer prognosis in each[19,20]. TNF-α may also play an important role in tumor initiation by stimulating the production of intracellular ROS that may damage DNA and lead to genomic mutations[21]. IL-6 is another prototypic inflammatory cytokine and may directly influence tumor growth by enhancing the proliferation and survival of malignant cells in multiple myeloma, non-Hodgkin's lymphoma, and hepatocellular carcinoma[22,23]. Both TNF-α and IL-1β have been found to activate hypoxic signaling pathways in human hepatoma cells[24], suggesting that these inflammatory cytokines influence tumor growth both indirectly, by

modulating the microenvironment, and directly, by regulating tumor cells. TGF-β has also been reported to affect the microenvironment and enhance tumor formation by suppressing the antitumor activity of T-cells, natural killer cells, neutrophils, monocytes, and macrophages that are involved in protective surveillance mechanisms[25]. Therefore, it is likely that the cytokines and growth factors produced during chronic inflammation have pleotropic effects on tumor formation and growth that involve both direct effects on tumor cells and indirect effects by promoting favorable conditions within the microenvironment.

Oxidative Stress

Activated immune cells are the primary source of increased levels of ROS (O_2^-, H_2O_2, OH, HOCL) that may broadly enhance tumorigenesis. Perhaps most directly, ROS can induce genotoxic damage that results in oncogenic mutational events[10]. In addition, increased intracellular ROS levels may induce the activation of redox-sensitive transcription factors that enhance tumor formation[26,27]. For example, the Forkhead box class O (FoxO) transcription factors are activated and translocated into the nucleus in response to increased levels of ROS through the c-Jun N-terminal kinase-dependent signaling pathway and then induce the expression of cellular proteins that serve as ROS scavengers and attenuate cellular damage[28–30]. FoxO transcription factors also regulate a wide variety of additional cellular functions, such as proliferation, apoptosis, and differentiation, that may promote tumorigenesis and cancer progression[31–34].

Hypoxia

Chronic inflammatory disorders are frequently associated with an increased incidence of anemia, which may result in increased tissue hypoxia. Several cytokines released during immune responses can inhibit both hematopoiesis and erythropoiesis[35]. For example, increased levels of circulating IL-6 may decrease serum iron levels and the subsequent production of red blood cells[36]. Although evidence directly implicating anemia as a cause of cancer is lacking, a nested case-control study in a large cohort of blood donors has demonstrated that a large proportion of patients with hematologic malignancies had anemia 2 to 3 years before diagnosis. A similar pattern of anemia has been observed in gastrointestinal cancer[37]. Therefore, relative tissue hypoxia arising from anemia may promote tumor initiation and progression, or alternatively, the suppression of normal erythropoiesis may be a sensitive and early indicator of tumor occurrence.

Tissue Repair and Regeneration

Chronic injury associated with inflammatory conditions, such as sclerosing cholangitis and inflammatory bowel disease, has been associated with an increased risk of cancer[15,16]. Tissue repair and regeneration may involve specific pathways, such as Notch, Wnt, and Hedgehog (Hh), that are required during normal embryonic development. Although these pathways are subsequently silenced in most tissues, they may be reactivated following tissue injury to promote repair. Moreover, aberrant activation of these pathways has been implicated in a large number of human cancers[38]. Therefore, dysregulation of pathways involved in tissue regeneration may promote carcinogenesis.

Chronic Inflammation and Normal Stem Cell Function

In addition to potential effects on carcinogenesis, several factors produced during chronic inflammation may directly affect normal stem cell function. For example, TNF-α has been found to induce neural stem cell proliferation and to inhibit differentiation into neuronal progenitor cells through nuclear factor κB signaling and increased expression of cell cycle regulators, including cyclin D1[39]. Furthermore, the gp130 protein, which is a part of the receptor of IL-6, has been reported to play a role in the self-renewal of hematopoietic stem cells[40].

ROS levels may also regulate normal stem cells, perhaps best exemplified by their effects on normal hematopoiesis[28,29]. Hematopoietic stem cells appear to have lower levels of ROS than their mature progeny, and this feature may maintain their self-renewal potential by inhibiting differentiation[41,42]. Moreover,

conditional inactivation of several genes, including the FoxO3 transcription factors, p53, and p38 mitogen-activated protein kinase, has been associated with increased ROS levels and the loss of hematopoietic stem cell function[43–45]. FoxO3 activation normally attenuates ROS levels via the ataxia telangiectasia mutated gene (ATM)[46], which mediates the cellular response to DNA and oxidative damage[47]. Accordingly, the loss of ATM also results in increased ROS levels and depletion of the hematopoietic stem cell pool[42,48]. It is likely that increased ROS levels within hematopoietic stem cells induce cellular damage and apoptosis. However, the loss of FoxO3 also results in increased proliferation, and the loss of cellular quiescence also likely contributes to decreased hematopoietic stem cell function[49]. In a similar manner, the loss of FoxO3 function results in reduced numbers and function of normal stem cells in the central nervous system[50]. Therefore, ROS levels may affect normal stem cells by modulating their survival or functional properties, which include self-renewal and differentiation.

Similar to oxidative stress, hypoxia may also regulate stem cell quiescence and self-renewal[51–53]. The cellular response to oxygen levels is regulated by the transcriptional activity of hypoxia-inducible factors (HIFs). Both hematopoietic stem cells and neural stem cells are thought to reside in regions of relatively low oxygen content[54]. These relatively hypoxic conditions lead to increased activity of HIF-1α and the expression of HIF-transcriptional targets, including FoxO3, that are important for the maintenance of the hematopoietic stem cell pool[55]. Therefore, HIF-1α activity may promote cellular quiescence and preserve self-renewal in response to hypoxia via such factors as FoxO3[49,56].

By their very nature, several pathways activated during tissue regeneration are required for normal stem cell function. During normal development, the Hh, Notch, and Wnt pathways play critical roles in stem cell fate decisions required for proper patterning and organogenesis. Moreover, these pathways have been implicated in the regulation of stem cell self-renewal and differentiation in many adult tissues, including hematopoietic tissues and the central nervous system[57].

Potential Role of Inflammation in Cancer Stem Cell Initiation, Regulation, and Function

Chronic inflammatory states may affect both carcinogenesis and normal stem cell function, and it is possible that the convergence of these activities contributes to the formation or regulation or both of CSCs. Increased intracellular ROS levels generated by immune effectors or inflammatory cytokines are a common feature of chronically inflamed tissues and may have multiple effects on CSCs. The self-renewal of normal hematopoietic stem cells is modulated by ROS levels, in part through the regulation of FoxO transcription factor activity[49]. A recent study using a mouse model of chronic myeloid leukemia (CML) has found that FoxO3 activity is required for the maintenance of leukemic stem cells[58]. Therefore, it is possible that increased ROS levels induced during chronic inflammation promote aberrant self-renewal through mediators, such as FoxO3. Moreover, increased intracellular ROS levels may induce DNA damage within CSCs which results in additional mutations that promote disease progression. Several adaptive mechanisms are normally activated in response to increased oxidative stress and may promote drug resistance in cancer[59–61]. In some diseases, CSCs have been found to show increased levels of ROS compared with their normal counterparts[41], and it is possible that these adaptive processes, including the activation of FoxO and ATM, are responsible for the relative drug resistance that has been attributed to CSCs.

Another hallmark of chronic inflammatory states is increased local levels of cytokines and growth factors, and accumulating data suggest that CSCs may be modulated by these factors. For example, IL-6 has been found in enhance the tumorigenicity and self-renewal of CSCs in glioblastoma[62,63]. Furthermore, TGF-β may regulate CML stem cells by regulating the activity of Akt signaling[58]. Therefore, soluble factors released by inflammatory cells may positively regulate CSCs. In addition, TGF-β is a well-recognized regulator of the epithelial-to-mesenchymal transition (EMT) in solid tumors, and this process is thought to play an important role in

the development of metastatic disease[64]. The EMT is characterized by the loss of epithelial markers (E-cadherin), the gain of mesenchymal markers (N-cadherin and vimentin), and the induction of the Snail family of transcriptional regulators, which result in increased cellular motility and invasive potential[65,66]. The EMT may represent a transitional state for epithelial cells which promotes their adaptation to cellular stress, and recent data suggest that cancer cells possessing features of the EMT have enhanced stem cell properties. In immortalized mammary epithelial or breast cancer cells, treatment with TGF-β or forced expression of Snail or Twist induces phenotypic CSC markers and enhances tumorigenicity both *in vitro* and *in vivo*[67,68]. Moreover, enhanced ROS levels can induce EMT, suggesting yet another mechanism by which chronic inflammation may induce the formation of CSCs[69,70].

Hypoxic conditions may play an important role in the regulation of CSCs[71]. Recent studies in brain tumors demonstrate that HIF-1α is active within CSCs located in the hypoxic niche[72]. Moreover, the activation of HIF-1 α maintains both an undifferentiated phenotype and self-renewal capacity[73]. In the bone marrow, hypoxic niches and HIF-1α play critical roles in the regulation of normal hematopoietic stem cells [71]. Recent reports demonstrate that the bone marrow environment filled with leukemic cells is hypoxic[74,75] and that HIF-1α is present in leukemic cells in patients with primary acute lymphoblastic leukemia[76]. Moreover, emerging data suggest that alterations in tumor cell metabolism affect both ROS and HIF-1α levels that may regulate CSCs. In AML, somatic mutations of isocitrate dehydrogenase have been identified, and the loss of the normal isocitrate dehydrogenase activity increases ROS levels and HIF-1α activity[77,78]. Similar mutations may occur during the transformation of myeloproliferative diseases to AML, suggesting that both ROS and HIF-1α may further alter CSC function during disease progression[79,80]. It is also possible that relative tissue hypoxia may enhance the metastatic potential of CSCs, as HIF-1α activation can promote the EMT directly by inducing the expression of Twist[81,82]. Therefore, hypoxic stress following tissue injury may promote the formation of CSCs and regulate their functional properties through multiple mechanisms.

Several processes regulating tissue repair may also be potentially subverted to alter normal stem cell function. For example, developmental signaling pathways may be necessary for tissue regeneration by modulating the activities of normal stem cells. However, during chronic injury, their sustained activation may lead to aberrant stem cell expansion or dysregulation of self-renewal and result in carcinogenesis[83]. For example, mutations within components of the Hh signaling pathway may lead to the development of skin cancers and brain tumors, suggesting that aberrant pathway activation induces the formation of cancer stem cells in each of these diseases[83]. Increased Hh signaling has also been identified within CSCs in CML, multiple myeloma, glioblastoma, and pancreatic cancer, and pharmacologic inhibition of the Hh pathway may prevent tumorigenicity, self-renewal, and metastatic potential[83]. Therefore, the inhibition of developmental signaling pathways may serve as novel strategies to inhibit CSCs.

Conclusion

Chronic inflammation is clearly associated with an increased risk of cancer. Although the precise events that lead to tumor formation are unknown, it is possible that specific factors, such as inflammatory cytokines, increased ROS levels, hypoxia, and the activation of developmental signaling pathways, play critical roles by regulating CSCs. These processes are likely to interact with one another in complex ways to ultimately affect both cancer formation and CSC functions, such as self-renewal, drug resistance, and metastatic potential. Further elucidation of the mechanisms by which these factors influence CSC may lead to improved strategies to prevent cancers and to the development of novel therapeutic targeting approaches.

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