HEMOCHROMATOSIS AND CANCER

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Most neoplastic disorders have a complex and multifactorial background, which may be influenced by a number of different determinants, among which is iron. Experimental, clinical and epidemiological investigations have shown that iron can influence the process of carcinogenesis in different ways: by catalysing the formation of mutagenic hydroxyl radicals, by suppressing host defence cells and by acting as an essential nutrient for proliferating tumour cells.

Experimental and human data support the hypothesis that iron overload is a risk factor for cancer in general and liver cancer in particular. In cirrhosis due to genetic haemochromatosis there is an increase incidence of hepatocellular carcinoma. The term hemochromatosis should refer to a unique clinicopathologic subset of iron overload syndromes that currently includes the disorder related to the C282Y homozygote mutation of the hemochromatosis protein HFE (by far the most common form of hemochromatosis).

In this article, we review the current information regarding our understanding of the relation between iron and the immune system, iron as a factor of carcinogenesis, epidemiology of several types of cancer relating to iron overload, in particularly using hemocromatosis, an iron overload model.

Iron and Immunity

A number of genes and proteins primary involved in iron homeostasis, namely in iron binding, transport and storage are now recognised to display related immunological functions.

Effects of iron overload include decreased antibody-mediated and mitogen-stimulated phagocytosis by monocytes and macrophages, alterations in T-lymphocyte subsets, and modification of lymphocyte distribution in different compartments of the immune system.[1]

Abbreviations: HH, hereditary hemochromatosis; HCC, hepatocellular carcinoma; CLD, chronic liver disease; HCV, hepatitis C virus; TfR, transferrin receptor; HNPCC, nonpolyposis colorectal cancer;

The poor ability of lymphocytes to sequester excess iron in ferritin may help to explain the immune system abnormalities in iron-overloaded patients. Iron overload as seen in hereditary hemochromatosis (HH) patients enhances suppressor T-cell (CD8) numbers and activity, decreases the proliferative capacity, numbers, and activity of helper T cells (CD4) with increases in CD8/CD4 ratios, impairs the generation of cytotoxic T cells, and alters immunoglobulin secretion when compared to treated hereditary hemochromatosis patients or controls.[1]

A variety of laboratory and clinical investigations have observed that one of the dangers of excessive iron is its ability to favor animal viral infections. The metal is essential for host cell synthesis of virions and can also impair defense cell function and increase oxidative stress. In both animal models and humans, viral infections cause upregulation of the iron withholding defense system. Factors that suppress the system enhance viral progression; factors that strengthen the system augment host defense.[2-7]

Withholding iron from potential pathogens is a host defense strategy. There is evidence that iron overload *per se* compromises the ability of phagocytes to kill microorganisms. Several hypotheses exist to explain the association of hemochromatosis with infection. A combination of mechanisms likely contributes to the increase in susceptibility to infection in these patients.[8]

The cells of the innate immune system, as part of a non-specific defense against infection, are equipped to express genes and proteins that can modulate iron homeostasis both at the cellular and the systemic levels. One central player in this modulation is hepcidin.[9] Hepcidin is a small protein comprised of 25 amino acids, synthesized in the liver. It was first described as a component of the innate immunity due to its antimicrobial activity.[10] Soon after, hepcidin was recognized as a key component in iron homeostasis, involved in maladies of iron overload or iron deficiency.[11-18]

The major mechanism of hepcidin function seems to be the regulation of transmembrane iron transport. Hepcidin binds to its receptor, protein ferroportin, which serves as a transmembrane iron channel enabling iron efflux from cells. The hepcidin-ferroportin complex is then degraded in lysosomes and iron is locked inside the cells (mainly enterocytes, hepatocytes and macrophages). This leads to lowering of iron absorption in the intestine and to a

decrease in serum iron concentration. Utilizing this mechanism, hepcidin regulates serum iron levels during inflammation, infection and possibly also in cancer. Under these conditions iron is shifted from circulation into cellular stores in hepatocytes and macrophages, making it less available for invading microorganisms and tumor cells.[19-21]

Iron and Cancer

Numerous laboratory and clinical investigations over the past few decades have observed that one of the dangers of iron is its ability to favour neoplastic cell growth. In both animals and humans, primary neoplasms develop at body sites of excessive iron deposits.[22]

Iron compounds were first reported to induce sarcomas in rats by Richmond in 1959.[23] Thereafter, several iron-induced carcinogenesis models were established, including the ferric nitrilotriacetate model by Okada *et al.*[24] Okada *et al.* demonstrated that phlebotomy after the administration of ferric nitrilotriacetate did not reduce the incidence of renal cell carcinoma. In addition showed that iron withdrawal at the promotion stage of carcinogenesis retarded tumor growth.[24] It was reported in 2008 by Zacharski *et al.* that iron reduction by phlebotomy was associated with lower cancer risk and mortality in patients with peripheral arterial disease.[25]

One should not underestimate the role of iron in carcinogenesis.

The metal is carcinogenic due to its catalytic effect on the formation of hydroxyl radicals, suppression of the activity of host defence cells and promotion of cancer cell multiplication.

Iron-induced oxidative stress results in two possible consequences: (1) redox regulation failure that leads to lipid peroxidation and oxidative DNA and protein damage; (2) redox regulation that activates a variety of reducing and oxystress-protective mechanisms via signal transduction. Both consequences appear to play a role in iron-induced carcinogenesis.[26]

Iron is an essential metal in mammals for oxygen transport by hemoglobin and for the function of many enzymes including catalase and cytochromes. However, the "free" or "catalytic" form of iron mediates the production of reactive oxygen species and induces oxidative stress. Serum "free" iron is observed in rare situations such as in severe hemochromatosis in which serum transferrin is saturated. However, it is known that superoxide can release "free" iron from ferritin and hemosiderin in the cell. "Free" iron is quite cytotoxic as well as mutagenic and carcinogenic. In humans, genetic hemochromatosis and asbestosis are two major diseases associated with iron-induced carcinogenesis. There is an increasing number of reports of an association between increased body iron stores and increased risk of cancer.

Hemochromatosis is not the only human disease with complications of iron overload-induced cancer. Persistent damage to hepatocytes reduces the production of hepcidin, which promotes iron absorption and deposition irrespective of the iron stores. Several studies on patients with chronic HCV have shown that hepatic iron overload is attributable to liver injury and that iron depletion improved serum aminotransferase levels.[27] Long-term iron depletion for chronic hepatitis C patients is a promising modality for lowering the risk of progression to hepatocellular carcinoma.[28,29]

Several epidemiological studies have suggested an association of endometriosis and ovarian cancer, demonstrating a high risk of ovarian cancer in women with a long-standing history of ovarian endometriosis.[30] Recent study revealed that ovarian endometriotic cysts are rich in catalytic iron, leading to increased oxidative DNA damage of the epithelia of those cysts.[31]

Hemochromatosis and cancer

Hereditary hemochromatosis is a genetic iron overload disorder that in the past was difficult to diagnose until the progressive accumulation of iron, mainly in the form of ferritin and hemosiderin, caused solid organ injury, particularly to the liver, heart, and endocrine pancreas (especially insulin-secreting β-cells). *HFE*, the gene responsible for hereditary hemochromatosis, is related to major histocompatibility complex class I proteins, and is mutated in hereditary hemochromatosis.[32] Two point mutations, C282Y and H63D, have been linked to the majority of disease cases.[33] Hereditary hemochromatosis is usually inherited in an autosomal-recessive manner.[34]

Major causes of death today in hereditary hemochromatosis are due to either hepatic failure with cirrhosis or hepatocellular carcinoma.[35] Indeed greater risk was shown for primary hepatocellular carcinoma in hemochromatosis patients.[36-38] In general, hepatocellular carcinoma is preceded by cirrhosis.

Hepatocellular carcinoma (HCC) is a common cause of death in patients with compensated cirrhosis.[38-41] Thus, it is important to identify patients at high risk of HCC, to increase the rate of early detection.

A role of HFE mutations as part of a genetic pattern promoting carcinogenesis has been evaluated in some studies, with somewhat discordant results. In a German study, C282Y heterozygosity was significantly more

common in 137 HCC cases with no history of HH versus 107 cirrhotic patients without HCC and 126 healthy controls. C282Y heterozygote HCC patients had significantly increased hepatic iron score in both HCC and non-tumourous tissue.[42]

Other studies reported that the prevalence of C282Y heterozygosity was increased above control levels in patients with HCC and alcoholic and virus-related liver disease.[43-45] An excess of the C282Y mutation, mostly in the heterozygous genotype, has also been reported in patients with HCC developed in non cirrhotic liver. In that study, Blanc and colleagues showed that mild iron overload is frequent and that in patients with HCC in non cirrhotic liver and iron overload, C282Y mutations are frequent and significantly increased compared to HCC in non cirrhotic liver without iron overload.[46]

Reports have indicated that patients with HH and cirrhosis are at high risk of developing liver cancer, the estimated probability being 200 times higher than in the general population.[47-49] However, when cirrhotic HH patients were compared with unmatched cirrhotic patients with cronic liver diseases of different etiology, no difference was found in the incidence of liver cancer.[50]

Several studies also did not find a relationship between HCC and mutations in the HFE gene. Cauza and colleagues evaluated the prevalence of HFE mutations in patients with HCC developed on cirrhosis of viral and alcoholic aetiology. The authors found that, except for C282Y homozygotes, HFE mutations did not increase the risk of HCC in patients with cirrhosis.[51] A prospective study of 133 consecutive cirrhotic patients without HH did not find an increased prevalence of HFE mutations in cirrhotic patients who developed HCC compared to cases without HCC[52]. Similarly, a study from Italy found that in patients with HCC in the absence of HH, the frequency of HFE mutations was not increased, compared to the controls. The authors concluded that mutations of the HFE gene do not play a significant role in the pathogenesis of HCC[53].

However recently Ezzikouri *et al.* showed that H63D carriage is increased among hepatocellular carcinoma moroccan patients, suggesting that it may confer an increased susceptibility to hepatocellular carcinoma even in a heterozygous state. On the contrary, HFE C282Y mutations did not contribute to hepatocellular carcinoma development.[54]

However Nahon *et al.* found that liver iron overload and C282Y mutation were associated with a higher risk of hepatocellular carcinoma in patients with alcoholic but not HCV-related cirrhosis.[55]

Iron has a clear pathogenetic role in the development of HCC in HH.

It has been suggested that iron plays a key role in the occurrence of hepatic cancer although, in most cases, it develops several years after iron depletion.[56-58]

The possible role of iron as a factor facilitating the development of liver cancer has been also suggested by the finding of increased iron content in the non-cirrotic livers of non-HH patients undergoing liver transplantation for hepatocellular carcinoma (HCC).[58]

The available studies about the role of HFE mutations in HCC in liver diseases other than HH indicate that C282Y heterozygosity may play a role in liver iron deposition and could contribute to hepatocarcinogenesis, possibly by playing a part in the immunogenetic pattern of the patient or through subtle changes in iron metabolism acting together with other cofactors.

A high incidence of cancers originating from other organs have been reported.[59-64] Increased cancer risks for extrahepatic neoplasms have been reported including that for hematologic, gastric, and colorectal tumors.[63-64]

It has long been debated whether excess iron also facilitates the occurrence of extrahepatic cancers in patients with or without HH. In comparison with the general population, a significantly higher risk of esophageal cancer and skin melanoma has been found in Danish HH patients,[65] and of lung and intestinal cancer in a series of English HH patients[66]; however, no increased risk of extrahepatic cancer was found in Australian HH patients against those with chronic liver diseases of different etiology.[67] In addition, an increased risk of colorectal and gastric cancers and hematologic malignancies were found in a large series of subjects heterozygous for HH.[68]

Hemochromatosis gene mutations are associated with male breast cancer.[69] Syrjäkoski *et al.* found a minor role for the HFE mutations C282Y and H63D in the causation of male breast cancer and prostate cancer. However carriers of both BRCA2 9346(-2)A-->G and an HFE mutation may be at an increased risk.[70]

About hematologic malignancies, the study of Veneri *et al.* did not support the evidence of an association between hemochromatosis gene mutations and iron overload in acute leukemia patients.[71] Ruiz-Argüelles *et al.* also arrived to the conclusion that HFE gene mutations were not risk factors for the development of leukemia in Mexican mestizos.[72] However it was found recently that there is an increased cancer risk in C282Y carriers likely due to higher iron levels in a multifactorial setting. In childhood acute lymphoblastic leukemia (ALL), there is an association of C282Y with a gender effect in two British populations. The childhood leukemia association possibly results from

elevated intracellular iron in lymphoid cells increasing the vulnerability to DNA damage at a critical time window during lymphoid cell development. Interactions of HFE with environmental and genetic factors, most of which are recognized, may play a role in modification of susceptibility to leukemia conferred by C282Y. Given the population frequency of C282Y and the connection between iron and cancer, clarification of the mechanism of HFE associations in leukemia and cancer will have strong implications in public health.[73] Viola et al. demonstrated a correlation between the presence of the H63D mutation and the occurrence of acute lymphoblastic leukemia in adult patients.[74]

Hereditary nonpolyposis colorectal cancer (HNPCC) is characterized by germline mutations in DNA mismatch repair genes. Homozygosity for the HFE H63D polymorphism seems to be a genetic modifier of disease expression in HNPCC. Understanding the mechanisms by which HFE interrelates with colorectal malignancies could lead to reduction of disease risk in HNPCC.[75]

Conditions causing high iron levels, such as hemochromatosis, are proposed risk factors for esophageal adenocarcinoma. Corley *et al.* conducted a case-control study of persons with a new Barrett's esophagus diagnosis, persons with gastroesophageal reflux disease (without Barrett's esophagus), and population controls. Subjects completed detailed examinations and assays for hemochromatosis mutations and serum iron stores. It was concluded that Barrett's esophagus was not associated with hemochromatosis gene defects.[76]

Fracanzani *et al.* studied a large series of patients with HH or non–iron-related cronic liver disease and found that the relative risk of developing both hepatic and extrahepatic malignancies was significantly higher in the HH patients who also had a higher prevalence of 2 independent primary cancers.[77]

Fracanzani *et al.* also concluded that the prospective study comparing HH patients and patients with non–iron-related chronic liver disease (CLD) unequivocally indicate that extrahepatic malignancies are also facilitated by excess body iron, thus confirming the data by Nelson *et al.*[78] on the risk of malignancies in HH heterozygotes.

Various experimental data suggest that iron can act as a cofactor in chemical carcinogenesis[79] but has no effect in the absence of a promoter. This may explain why liver cancer developed only in cirrhotic HHpatients, in whom nodular regeneration acts as a promoter.[80-82]

Similarly, in the case of extrahepatic malignancies, subliminal stimuli may also induce neoplastic transformation in nonliver tissues containing increased iron stores.

It was also found that HFE mutations can have a protection factor against some virus. HFE mutated macrophages may be better equipped to protect from HIV-1 infection, compatible with the description of a long survival in a patient with AIDS and hereditary hemochromatosis.[83]

HFE could be considered a candidate modifier gene of viral-related neoplasia such as cervical carcinoma possibly by a dual role on iron metabolism and immunological system. Cardoso *et al.* found a significantly lower risk of development of cervical neoplasia in H63D carriers but no association with C282Y.[84]

Conclusions

The present data clearly indicate that iron removal therapy should be started as soon as possible in HH patients not only to reduce the risk of cirrhosis and subsequent liver cancer, but also to reduce the risk of extrahepatic malignancies. Whether this approach should be extended to patients with no signs of HH but minor increases in body iron stores still needs to be evaluated.

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