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Translational Oncogenomics

Evidence for the Influence of the Iron Regulatory MHC Class I Molecule HFE on Tumor Progression in Experimental Models and Clinical Populations

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ABSTRACT: Proteins involved in iron regulation are modifiers of cancer risk and progression. Of these, the HFE protein (high iron gene and its protein product) is of particular interest because of its interaction with both iron handling and immune function and the high rate of genetic polymorphisms resulting in a mutant protein. Clinical studies suggest that HFE polymorphisms increase the risk of certain cancers, but the inconsistent outcomes suggest a more nuanced effect, possibly interacting with other genetic or environmental factors. Some basic science research has been conducted to begin to understand the implications of variant HFE genotype on cancer, but the story is far from complete. In particular, putative mechanisms exist for HFE to affect tumor progression through its role in iron handling and its major histocompatibility complex class I structural features. In this review, the current understanding of the role of HFE in cancer is described and models for future directions are identified.

KEYWORDS: iron, HFE, hemochromatosis, overload, tumor progression

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Introduction

Cancer is one of the leading causes of mortality in the United States.[1] Tumors require iron and have increased ferritin and transferrin receptor 1 (TfR1) expression compared to normal tissue and stroma.[2] Intracellularly, iron regulates several key cell cycle proteins including p53, p27kip1, cyclin D1, cdk1, and p21CIP1/WAF1.[3–5] Proteins involved in iron regulation are modifiers of cancer risk and progression. For example, in clinical specimens, overexpression of iron import proteins was associated with increased progression of colorectal cancer[6] and malignant progression of esophageal adenocarcinoma and risk of metastasis.[7] In breast cancer, cell lines with aggressive, mesenchymal phenotypes expressed more ferritin, transferrin, TfR, and iron regulatory proteins compared to less aggressive lines.[8] We have reported that elevated ferritin in serum is associated with poor prognosis in breast cancer and that ferritin may have a direct impact on the tumor.[9] In addition, ferroportin levels are linked to tumor progression in breast cancer such that impaired iron export is linked to a more aggressive phenotype.[10] Another study in MAL-12 murine hepatocytes showed that transforming growth factor β1, a known promoter of tumor aggression, induces epithelial to mesenchymal transition (EMT) through inhibition of ferritin heavy chain, increasing the labile iron pool and generating reactive oxygen species (ROS).[11] EMT is a widely studied phenomenon believed to be associated with the metastatic progression of tumor cells as well as certain developmental migratory processes. We have recently reported that decreased expression of H-ferritin in glioblastoma multiforme increases the vulnerability of these cells to therapeutic strategies.[12] In the field of metastasis, the protein n-myc–downregulated gene 1, the iron-related metastasis suppressor, links iron metabolism to cancer progression.[13] The intent of the present review is to describe the relationship between cancer progression and the

most common gene variant in Caucasians: HFE (high iron protein). The HFE protein is involved in iron regulation and immune system function and thus sits at the intersection of two major physiological processes in cancer biology. HFE polymorphisms are extremely common in the United States, with prevalence of 5.4% for C282Y and 13.5% for H63D and higher numbers in Caucasians (6.2% and 15.1%, respectively).[14] Because of the prevalence of these variants, focusing on HFE provides the possibility of understanding the special risks and treatment considerations that may apply to cancer patients who are carriers of variant HFE alleles.

The HFE protein. The HFE protein is an atypical class I major histocompatibility complex (MHC) molecule that acts on iron homeostasis through its interaction with β2 microglobulin (β2 m) and the TfRs (1 and 2). Normal HFE function limits iron intake into the cell, preventing iron overload (Fig. 1). The mutant form of this protein has long been thought to be related to hereditary hemochromatosis (HH) in humans,[15] although the penetrance of HH relative to the gene variant is very low.[16] The H63D polymorphism translocates to the cell surface but fails to participate in the interactions with the TfR1, and the C282Y polymorphism cannot interact with β2 m, preventing its surface translocation.[17] An illustration of the normal function of HFE in a typical parenchymal cell is given in Figure 2. Furthermore, HFE polymorphisms have been shown to directly affect immune function. For example, peripheral blood mononuclear cells from patients with variant HFE expression have lower levels of surface MHC I molecule expression, which are important presentation molecules affecting leukocyte activation in cancer.[18–20]

An exploration of the relationship between HFE genotype and cancer must, of course, include a consideration of the role of iron in cancer. The relationship is considered complex and is incompletely understood, yet iron metabolism and cancer progression are clearly linked.[21] While a detailed examination of the relationship between iron and cancer is beyond the scope of this review, the authors refer the reader to previous reviews on the subject and supply here a cursory overview.[22,23] The favored mechanism for linking iron and cancer is through the generation of ROS, which in turn induce a more aggressive tumor phenotype.[24,25] Supporting this mechanism, it has been shown that HFE knockout mice are at greater risk for oxidative damage, and the intake of iron exacerbates this phenomenon.[26] However, we will explore this concept in greater detail and provide evidence that the mutant HFE protein itself may impose altered cellular phenotype that alters cancer biology.

The concept that HFE mutant protein may directly impact cancer phenotype would not exclude a relationship with iron. In clinical studies, iron loading has shown a modest association with increased overall cancer risk, and specifically, lung cancer risk.[8,27] In addition, patients with HH, who experience severe iron overload, showed a risk of liver cancer over two hundred times greater than matched controls.[28] Conversely, regular blood donors, who have diminished iron load, have decreased overall rates of cancer.[29] Studies of spontaneous tumorigenesis in rats found that iron loading led to increased rates of tumor formation.[30–32] By the same token, feeding mice an iron-deficient diet results in smaller tumors in a xenograft model using three different cancer cell lines.[33] Experimentally, the iron chelator deferoxamine blocks the proliferation of glioma and neuroblastoma cells, supporting the manipulation of iron balance as a therapeutic approach in some cancers.[34,35] In cell culture, iron loading in Caco-2 and SW480 cells led to increased proliferation and loss of E-cadherin expression, suggesting EMT.[6] More recently, a 2011 study in several cell culture models found that suppression of HFE or β2m, which binds to HFE and facilitates trafficking, caused a reversal of EMT.[36]

Taken together, these studies highlight how the proteins of iron metabolism can modify tumor aggression and are thus potential targets of interest in the development of therapeutics. Although both H63D and C282Y fail to produce a properly functioning protein and are associated with increased iron uptake, cell culture studies have shown that these variants can impact cancer phenotype in dramatically different ways,[37] supporting the proposition in this review that the HFE impact on cancer biology is not limited to its impact on iron homeostasis.

While much of the focus on HFE and cancer has been on its connection to iron metabolism, as an MHC molecule, it is not surprising that HFE interacts with the immune system. For example, patients with C282Y homozygous genotype have been shown to have significantly lower total lymphocytes and CD4+ lymphocytes specifically compared to control subjects.[38] Also, HFE knockout mice have been shown to induce expression of lipocalin 2 (LCN2), an immune-signaling molecule.[39] Given that LCN2 has been implicated in tumorigenesis of leukemias and both tumorigenesis and metastasis in breast tumors, this is one possible mechanism for HFE to modulate tumor aggression.[40–42] The interaction of HFE with β2m may also have immunological consequences.[43,44] Given that β2m in the cerebrospinal fluid is associated with leptomeningeal spread of cancers, a relationship between central nervous system metastasis and HFE is feasible.[45] Lastly, analysis of patient plasma samples using a biochip array analyzer revealed decreased L-selectin, increased E-selectin, and increased intracellular adhesion molecule 1 in HH patients, suggesting that immune response is affected in the context of HFE mutation by way of altered adhesion molecules.[46] This is important to the progression of cancer in two ways: First, any disruption in the migration of leukocytes to the tumor would be expected to affect the tumor microenvironment and limit the ability of these cells to influence tumor growth. Second, at the metastatic stage, circulating tumor cells have been shown to mimic leukocyte behavior and utilize the selectin-mediated leukocyte adhesion cascade to infiltrate tissues.[47–49]

In addition to the impairment of protein function, HFE mutations may also alter cell biology due to the stress of producing and breaking down large quantities of extraneous protein. In our cell culture and animal models, the C282Y

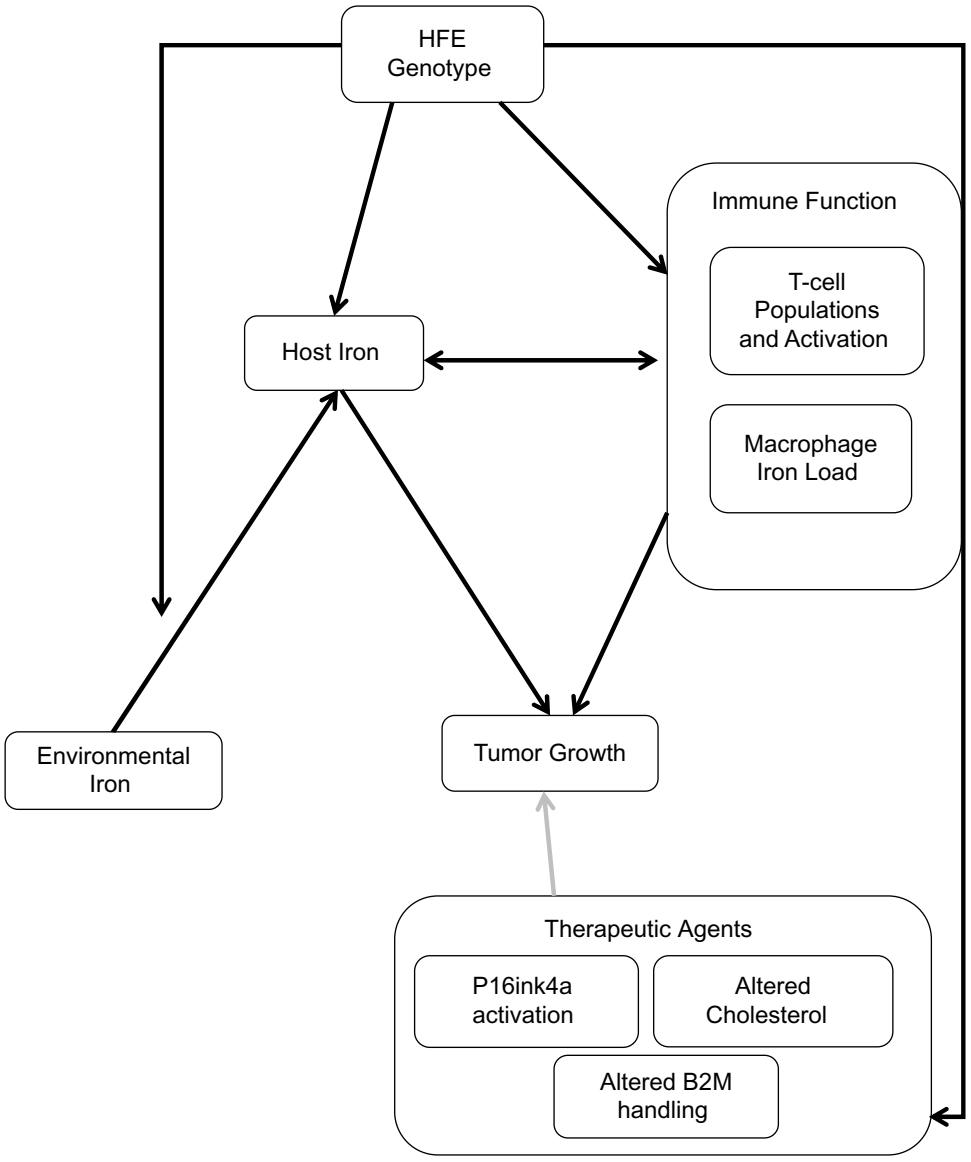

Figure 1. Conceptual framework of HFE as it relates to cancer. HFE genotype has been shown to affect both iron load and immune function. Although the relative importance of each cell type remains to be tested, some combination of these factors appears to affect tumor growth across a variety of cancer types. In addition, it is expected that HFE interacts with environmental factors, particularly dietary iron, to yield the observed effect on cancer risk and tumor progression. Lastly, HFE variants have been shown to affect the handling of β2m, the response to cholesterol-altering medications, and the levels of P16ink4a. These findings strongly suggest that HFE mutations will affect the efficacy of therapeutic interventions. Therefore, taking HFE genotype into account may one day provide valuable information on prognosis and could lead to more efficient application of pharmacologic interventions.

and H63D mutations have both been shown to create ER stress and activate the unfolded protein response (UPR).[50,51] In addition, C282Y HFE has been shown to create intracellular aggregates, which could affect cell signaling and survival through the binding sites on these aggregates.[50] UPR is known to affect cellular apoptosis and may contribute to promoting the survival of tumor cells.[52] Another mechanism by which HFE mutation–induced UPR could affect tumor spread is through effects on MHC I expression at the cell surface, altering the immune response to the cancer cells.[53]

Specifically, HFE C282Y mutant patients had peripheral blood mononuclear cells with reduced surface expression of β2m-assembled MHC class I molecules and increased free MHC class I molecules. Within the cell, the C282Y mutants had fewer free class I MHCs and more β2m-assembled class I molecules.[18]

In support of the ability of HFE to affect the immune system, it has been found that hepcidin response to inflammation depends on HFE and the TfR2.[54] Hepcidin normally sequesters iron in response to inflammation, which limits access to iron by microbes and should alter iron availability for neoplasms. In mice with knocked down HFE, TfR2, or both, the levels of hepcidin and the downstream iron sequestration were hindered.

Although this review focuses on HFE polymorphisms because of their high prevalence in the general population, there

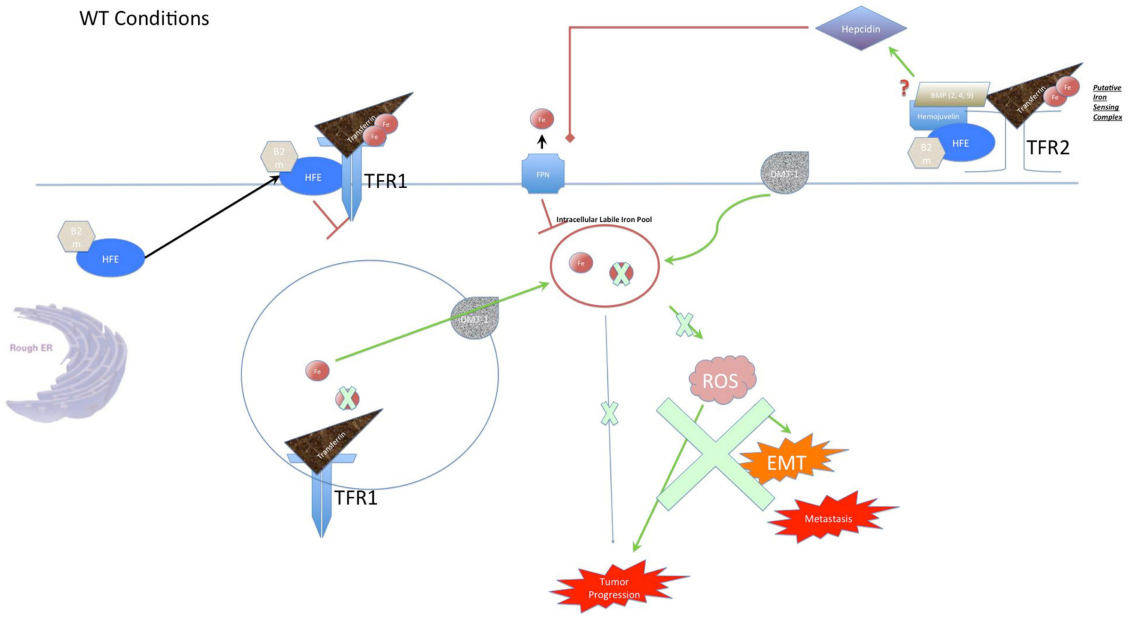

Figure 2. Function of the HFE protein in iron metabolism. When HFE is functioning normally, it associates with β2m and transits to the membrane, where it complexes with TfRs. At TfR1, it competes with transferrin to limit the rate of iron uptake into the cell, promoting a homeostatic level of iron load. Iron is taken into the cell when the entire TfR1 complex is endocytosed, and the pH of the compartment is acidified to the point where the iron dissociates from transferrin. From here, iron can pass through divalent metal transporter 1 (DMT-1), where it enters the cytoplasm. From here, the iron storage protein ferritin normally sequesters the free iron to limit its unwanted reactivity (not shown). For completeness, the diagram also shows the normal role of HFE in forming a complex with TfR2, which is thought to perform whole-body iron sensing in hepatocytes. In this way, host HFE is partially responsible for the proper secretion of Hepcidin, which suppresses the iron export protein ferroportin and encourages cells to retain iron intracellularly. Importantly, the regulation of iron load by HFE prevents an excess of ROS (generated via the Fenton reaction), which in turn are linked to EMT and metastasis. Furthermore, the limitation of iron supply prevents the cell from properly undergoing the cell division and metabolism that a progressing tumor would demand. Disruption of HFE function in the case of variant H63D genotype leads to iron overload because the HFE is unable to perform its normal function at TfR1. This leads to iron overload in the cell, which can lead to tumor progression and metastasis through increased stress on the endoplasmic reticulum, generation of ROS, and the increased availability of iron for cell metabolism and division. As the diagram indicates, this excess of ROS causes cellular damage and promotes EMT, leading to greater metastatic propensity. In addition, the decrease in interaction with TfR1 is thought to promote an interaction with TfR2 in hepatocytes, causing a systemic increase in hepcidin and suppression of ferroportin. Disruption of HFE function in the case of variant C282Y has similar effects to H63D in many ways. The key difference is that the mutation prevents the association of HFE with β2m, which prevents it from localizing to the cell surface at all. This would be expected to have similar effects on TfR1, since there is a loss of function in both cases. However, several lines of evidence show that the two mutations have divergent effects, so there must be a more nuanced effect on the cell than a simple loss of TfR1 blockade.

is also evidence that the HFE gene undergoes copy number alterations in many cancers.[55,56] If these copy number alterations affect mRNA levels of Wild Type (WT) or polymorphic HFE, the evidence suggests that they will affect cellular iron handling and immune presentation. Specifically, increased levels of WT HFE would be expected to limit iron uptake in cancer cells and decreased levels would lead to iron overload. This question has not been specifically studied, and future investigation would provide insight into the ability of HFE to affect tumor progression.

The effects of HFE on tumor progression have the potential to affect clinical practice not only as a risk factor but also HFE appears to modulate the response of cancers to standard therapeutic regimens. The population studies we will review in the rest of this document will focus on evaluating if HFE genotype is a risk factor for various cancers, only because there is a considerable knowledge gap on the impact of HFE genotype on therapeutic response. There is enough evidence to suggest that HFE is capable of affecting therapeutic response. For example, a 2006 study showed that expression of HFE reduces the effect of doxorubicin on proliferation in breast cancer cells.[57] In addition, a 2011 study conducted in our laboratory revealed that the C282Y mutation in HFE induced an elevation in p16INK4A, which was associated with treatment resistance in both neuroblastoma and astrocytoma cell lines.[58] One potential mechanism to explore further is the interaction between β2m and HFE, because β2m manipulation has been shown to affect therapeutic response.[59] Another mechanism by which HFE is likely to affect tumor progression and therapeutic response is by its effects on cholesterol and sphingolipid metabolism.[60] In particular, it is noteworthy that neuroblastoma cells with the C282Y variant of HFE exhibit improved survival in the presence of sphingosine kinase inhibitors, as this mechanism is already recognized as a chemotherapeutic strategy. Given the prevalence of HFE polymorphisms in the population, in the emerging

era of personalized medicine, considering HFE variants has a strong likelihood to improve patient care.

Clinical Studies

The data on whether HFE mutations are clinically important in cancer progression are quite variable, suggesting that non-genetic factors are affecting the penetrance of HFE genotype. HFE has shown to affect response to environmental stimuli in other contexts, so there is reason to believe that its effects would interact with the environment in tumor progression (n = 518).[61] Intuitively, dietary habits and iron content of drinking water could impact iron availability. In support of this idea, women have onset of iron overload about two years later than men in cases of hemochromatosis, and phlebotomy appears to be protective against the development of cancer, suggesting that removal of iron in blood is a significant modifier of these processes.[29,62] In breast cancer, Abraham and colleagues found no effect of H63D or C282Y on incidence, although a trend was seen toward more C282Y alleles in aggressive cases (n = 688 cancer patients, 724 matched controls).[63] In cervical cancer, H63D has been found to be associated with a lower risk (n = 346 samples, 201 with cervical cancer).[64] In contrast to these findings, H63D has been found to be associated with an increased risk of gastric cancer and with an increased risk of hepatocellular carcinoma (HCC; n = 365 gastric adenocarcinoma with 1284 matched controls, n = 100 cirrhosis patients, 100 HCC patients, and 100 controls).[65,66] A study comparing 100 consecutive oncology patients in central Alabama with 318 healthy controls found no significant differences in HFE variant allele frequency between the two populations.[67] This study argues against a large effect across several cancer types, but it was not sufficiently powered to detect more subtle differences in specific cancers. As the studies below will show, the literature shows both positive and negative results, either an absence of effect or an exacerbation of cancer risk or severity with HFE variant genotype. Thus, these data suggest there is a relationship between HFE genotype and cancer. The clinical studies evaluating the relationship between H63D and cancer have been summarized in Table 1, and the studies evaluating C282Y and cancer have been summarized in Table 2.

Leukemia

The available evidence suggests a possible effect of HFE genotype on acute leukemias, particularly in childhood. However, more recent studies have found that this interpretation may be influenced by nearby genes that segregate with HFE.

Initially, a 1999 case-control study by Dorak and colleagues in the United Kingdom found a male-specific association between HFE C282Y and childhood acute lymphocytic leukemia (ALL) (n(ALL) = 117, n(control) = 135).[68] In contrast, a small study of 36 acute myelocytic leukemia (AML) patients and 108 blood donor controls did not find an increased HFE variant allele frequency in AML patients.[69] A later Finnish study found no significant differences in HFE allele frequency in AML, ET, and AML in their populations, although they may have used too few subjects to detect subtle differences (232 total patients).[70] Similarly, a 2005study did not find differences in HFE gene variant allele frequencies in adult acute leukemia cases compared with the general population (n = 82).[71] However, HFE genotype (H63D and C282Y) has been found to be associated with increased risk of childhood ALL in females (n = 163 ALL, 995 controls).[72] It must be noted that the childhood ALL risk finding for C282Y was considerably weakened when it was examined in a more detailed study of polymorphisms in that chromosomal region. This argues for a contribution of nearby HLA genes and may suggest a smaller or noncontributory role for HFE in leukemia, but the animal and basic science data specific to HFE manipulation suggest that it has some effect. A follow-up study suggests that more statistical power is needed to determine if the effect is meaningful (n = 117 ALL, 414 newborn controls).[73] Finally, a 2013 study conducted in Spain did not find an association between HFE variant alleles and childhood ALL, which disagreed with earlier findings (see above) (n = 475 patients, 179 controls).[74] In toto, the evidence does not strongly support a relationship between HFE genotype and acute leukemias, especially in adults. The childhood effect warrants further investigation, and confounding factors will need to be considered going forward.

Breast Cancer

Breast cancer is a well-studied cancer that has already been linked to iron metabolism and inflammation.[9,75] Consistent with this is strong evidence for an effect of HFE variant genotype on breast cancer risk and prognosis. A 2004 study conducted on 168 patients and 169 matched controls in Tennessee showed an increased prevalence for C282Y polymorphisms in breast cancers than in healthy controls.[63,76] A 2005 study did not find differences in HFE gene variant allele frequencies in breast cancer cases compared to the general population, but did find that C282Y was associated with greater frequency in patients with greater lymph node involvement (n = 688 patients, 724 matched controls).[63] In 2006, a Turkish study compared 176 breast cancer patients to 200 healthy volunteers, finding no cases of C282Y in any of the studied patients and reporting a significantly increased frequency of H63D in breast cancer patients (39/176 versus 28/200, P = 0.02).[77] This is a notable example of regional effects on such studies, because an absence of C282Y would be quite rare in a European or North American population but is not surprising in a Middle Eastern population. Although a 2011 study conducted in Brazil found that H63D was associated with p53 mutations in breast cancer, it did not find an increased prevalence of HFE polymorphisms in breast cancer patients or a direct diagnostic or prognostic application for HFE genotype in this context (n = 68 patients, 85 controls).[78] A 2013 Norwegian study comparing 292 C282Y homozygotes

Table 1. Clinical studies of H63D and cancer have found, in general, either no effect or an increased risk of cancer for patients with H63D mutations. The finding that H63D appears to be protective in cervical cancer stands out and suggests a need for further study.

CANCER	REGION	H63D FINDING	SOURCE
Adult acute leukemia	Italy	No differences in frequency compared to general population	Veneri et al., 2005
ALL	United Kingdom	Increased Risk of childhood ALL in females	Dorak et al., 2009
ALL (Childhood)	Spain	No association between HFE variant Alleles and Childhood ALL	Rodriguez-Lopez et al., 2013
AML	Spain	No differences in frequency compared to blood donor controls	Gimferrer et al., 1999
AML	Finland	No significant differences in HFE allele frequency compared to general population	Hannuksela et al., 2002
Breast Cancer	Germany	No differences in frequency compared to general population	Abraham et al., 2005
Breast Cancer	Turkey	Significantly increased frequency of H63D in breast cancer patients compared to controls	Gunzel-Ozcan et al., 2006
Cervical Cancer	Portugal	Decreased Risk, later onset of cervical lesions	Cardoso et al., 2006
Colorectal Cancer	USA	No increased risk for colorectal adenoma in women	Chan et al., 2005
Colorectal cancer	Spain	No significant difference in HFE allele frequency compared to blood donor controls	Altes et al., 1999
Colorectal cancer	Poland and Australia	H63D homozygosity associated with significantly increased risk of colorectal cancer and earlier age of onset in patients with mismatch repair gene mutations	Shi et al., 2009
Colorectal cancer	USA	Significant increase in the risk of canger in patients with either HFE allele after adjusting for race, iron intake, red meat consumption, and NSAID use.	Shaheen et al., 2003
Colorectal cancer, breast cancer in women, prostate cancer	Australia	No increased risk noted	Osborne et al., 2010
Gastric Cancer	Europe (10 countries)	Increased Risk (Particularly Non-cardia site, Intestinal Histologic Subtype)	Agudo et al., 2013
Glioma	Italy	Significantly higher rate of H63D in high-grade gliomas compared to controls	Martinez di Montemuros et al., 2001
Hepatocellular Carcinoma	Italy	No increase in HFE mutations in HCC	Racchi et al., 2002
Hepatocellular Carcinoma	Sweden	20 fold increase in rates*	Elmberg et al., 2003
Hepatocellular Carcinoma	Spain	All H63D homozygotes did not have cirrhosis	Lauret et al., 2002
Hepatocellular Carcinoma	Egypt	Increased frequency of H63D in HCC cases compared to the general population in both alcoholic cirrhosis and hepatis C cohorts	Gharib et al., 2011
Hepatocellular Carcinoma	France	No significant diffrences in allele frequency between patients with cirrhosis and HCC and patients with cirrhosis without HCC	Boige et al., 2003
Hepatocellular Carcinoma	Spain	H63D was associated with risk of HCC in cirrhotic patients	Ropero et al., 2007
Male Breast, Prostate	Finland	No significant differences in H63D frequency compared to general population	Syrjakoski et al., 2006
Non-small-cell lung cancer	USA (Cooperative Human Tissue Network)	No evidence that NSCLCs select for HFE mutation	Muller et al., 2005
Several Types	Alabama, USA	No significant differences in HFE variant allele frequency compared to healthy controls	Barton et al., 2004

Note: *Elmberg et al[94] only examined patients with HH and did not assess which HFE polymorphisms were present.

with 62,568 controls from the HUNT 2 population screening study found that C282Y homozygosity was not associated with an increased risk of breast cancer, although it found increased risks of other cancer types.[79] Therefore, the evidence is suggestive of a risk but not totally conclusive, and there may be ethnic differences or gene–environment interactions that explain the discrepancy in results among different studies. In particular, it would be useful to fully understand the effects of dietary iron intake and iron loss through blood donation and menstruation.

Table 2. Clinical studies of C282Y and cancer similarly show either no effect or increased risk of cancer for patients with C282Y mutations. Going forward, controlled studies in disease models will help separate HFE effects from other genetic and environmental influences.

CANCER	REGION	C282Y FINDING	SOURCE
Adult acute leukemia	Italy	No differences in frequency compared to general population	Veneri et al., 2005
ALL	United Kingdom	Increased Risk of childhood ALL in females	Dorak et al., 2009
ALL (Childhood)	United Kingdom	Male-specific association between C282Y and ALL	Dorak et al., 1999
ALL (Childhood)	Spain	No association between HFE variant alleles and Childhood ALL	Rodriguez-Lopez et al., 2013
ALL (Childhood)	Poland	Examination of polymorphisms in the chromosomal region near HFE considerably weakened the power of the C282Y association with Childhood ALL. Further studies are needed	Sikorska et al., 2011
AML	Spain	No differences in frequency compared to blood donor controls	Gimferrer et al., 1999
AML	Finland	No significant differences in HFE allele frequency compared to general population	Hannuksela et al., 2002
Breast Cancer	Germany	No differences in frequency compared to general population, greater frequency in patients with lymph node involvement.	Abraham et al., 2005
Breast Cancer	Tenessee	Increased prevalence of C282Y mutations in breast cancer cases compared to healthy controls	Kallianpur et al., 2004
Cervical Cancer	Portugal	Not associated (no increase or decrease)	Cardoso et al., 2006
Colorectal and Primary Liver Cancer	Norway	Increased risk of colorectal cancer and primary liver cancer in C282Y homozygotes.	Asberg et al., 2013
Colorectal Cancer	Australia	Heterozygosity not associated with risk, site, or tumor stage	Macdonald et al., 1999
Colorectal Cancer	USA	No increased risk for colorectal adenoma in women	Chan et al., 2005
Colorectal cancer	Spain	No significant difference in HFE allele frequency compared to blood donor controls	Altes et al., 1999
Colorectal Cancer	Several (Meta-analysis)	C282Y mutation significantly associated with colorectal cancer in caucasians	Chen et al., 2013
Colorectal cancer	USA	Significant increase in the risk of cancer in patients with either HFE allele after adjusting for race, iron intake, red meat consumption, and NSAID use.	Shaheen et al., 2003
Colorectal cancer, breast cancer in women, prostate cancer	Australia	C282Y homozygotes were significantly more likely to contract colorectal cancer and (in females) breast cancer compared to WT individuals (no increased risk for prostate cancer)	Osborne et al., 2010
Hepatocellular Carcinoma	Italy	No increase in HFE mutations in HCC	Racchi et al., 2002
Hepatocellular Carcinoma	Europe	C282Y is associated with hepatocellular carcinoma	Jin et al., 2010
Hepatocellular Carcinoma	Sweden	20 fold increase in rates*	Elmberg et al., 2003
Hepatocellular Carcinoma	Spain	C282Y mutations were significantly more common in HCC + alcoholic cirrhosis than in alcoholic cirrhosis without HCC	Lauret et al., 2002
Hepatocellular Carcinoma	France	No significant diffrences in allele frequency between patients with cirrhosis and HCC and patients with cirrhosis without HCC	Boige et al., 2003
Hepatocellular Carcinoma	Italy	Increased prevalence of C282Y in HCC patients compared to healthy controls, trends toward an interaction with alcohol exposure and hepatitis virus markers.	Fargion et al., 2001
Hepatocellular Carcinoma	Germany	C282Y heterozygosity was significantly higher in HCC cases than control individuals	Hellerbrand et al., 2003
Hepatocellular Carcinoma	France	C282Y and iron load were associated with HCC risk in alcoholic cirrhotic patients but not HCV-related cirrhotic patients	Nahon et al., 2008
Hepatocellular Carcinoma	Spain	C282Y was not associated with increased risk of HCC in cirrhotic patients	Ropero et al., 2007
Hepatocellular Carcinoma	United Kingdom	C282Y was associated with an increased risk of HCC	Willis et al., 2000

(*Continued*)

Table 2. (*Continued*).

CANCER	REGION	C282Y FINDING	SOURCE
Male Breast, Prostate	Finland	No significant differences in C282Y frequency compared to general population	Syrjakoski et al., 2006
Non-small-cell lung cancer	USA (Cooperative Human Tissue Network)	No evidence that NSCLCs select for HFE mutation	Muller et al., 2005
Ovarian	Canada	Increased both risk and aggressiveness	Gannon et al., 2010
Several Types	Alabama, USA	No significant differences in HFE variant allele frequency compared to healthy controls	Barton et al., 2004

Note: *Elmberg et al[94] only examined patients with HH and did not assess which HFE polymorphisms were present.

Cervical Cancer

Although the research on HFE polymorphism in cervical cancer is limited, it is particularly interesting because it is a rare (perhaps unique) example of HFE variant genotype conferring a protective effect against cancer. Specifically, H63D carriers were at lower risk of developing cervical neoplasia, and they had a later onset of cervical lesions compared to controls. In this study, C282Y was not associated with cervical cancer progression ($n = 346$ total, 201 with cervical cancer).[64] This result suggests that HFE polymorphisms may be able to both antagonize and promote tumor progression depending on the carcinogenic mechanism of cancer. In this case, the strong association between human papilloma virus and cervical cancer may explain the divergent effect of HFE. If HFE H63D is disrupting some aspect of the viral process, it may be acting against the onset of cervical cancer before neoplastic changes ever set in.

Colorectal Cancer

Probably because of the known effects of HFE on intestinal iron absorption, the relationship between HFE genotype and colorectal cancer has been well studied.[80] In an Australian case-control study, HFE C282Y heterozygosity was not found in higher frequency in colorectal cancer cases (relative risk = 0.90). There was no association between heterozygosity of C282Y and tumor site or stage, either ($n = 229$ patients, 228 matched controls).[81] The same year, a study comparing 116 colorectal cancer cases to 108 blood donor controls found no significant difference in allelic frequency between the two groups.[82] However, an American study examining the relationship between colon cancer and HFE genotype found a significant increase in risk of colon cancer in patients with either H63D or C282Y alleles (odds ratio [OR] 1.4, 95% confidence interval [CI] 1.07–1.87) after controlling for several confounding variables including race, iron intake, red meat consumption, and nonsteroidal anti-inflammatory drug use ($n = 475$ patients, 833 controls).[83]

A study in women showed no increased risk for colorectal adenoma with HFE gene polymorphisms ($n = 527$ cases, 527 matched controls).[84] A 2009 study of Polish and Australian cohorts featuring 362 patients with mismatch repair gene mutations found that HFE H63D homozygosity was associated with a significantly increased risk of colorectal cancer (HR = 2.93, $P = 0.007$) and that H63D homozygous individuals had an earlier age of onset (44 years versus 50 years of age, $P < 0.05$).[85] A large prospective study conducted in Australia derived from the Melbourne Collaborative Cohort Study found that C282Y homozygotes were about twice as likely to contract colorectal cancer (HR 2.28, 95% CI 1.22–4.25, $P = 0.01$) and, in females, breast cancer (HR = 2.39, 95% CI 1.24–4.61, $P = 0.01$) compared to individuals with WT HFE. They found no evidence for increased risk of prostate cancer, nor did they see an increased risk of cancers in compound heterozygotes (with H63D and C282Y alleles) ($n = 28{,}509$).[86]

The previously mentioned 2013 Norwegian study found that C282Y homozygosity was associated with an increased risk of colorectal cancer and primary liver cancer in men (colorectal cancer hazard ratio 3.03, hazard ratio 3.03, 95% CI 1.17–7.82, $P = 0.022$; C282Y liver cancer hazard ratio 54.0, 95% CI 2.68–1089, $P = 0.009$; $n = 292$ homozygotes, 62,568 other subjects).[79] Most recently, a 2013 meta-analysis showed that the C282Y polymorphism was significantly associated with colorectal cancer in Caucasians (OR 2.00, 95% CI 1.32–3.04) (pooled $n = 7588$ colorectal cancer cases, 81,571 controls).[87] Taken together, the available studies favor the conclusion that HFE polymorphisms are associated with increased risk of colorectal cancer, but this association may be limited to specific populations and thus possibly dietary habits.

Gastric Cancer

Although the evidence is limited, there is reason to believe that the HFE polymorphism H63D is a risk factor for gastric cancer. Specifically, a study conducted within the European Prospective Investigation into Cancer and Nutrition showed an increased risk of gastric cancer, particularly the noncardia site and intestinal histological type, in patients with H63D ($n = 365$ patients, 1284 controls).[65]

Glioma

As with gastric cancer, studies examining the link between HFE and glioma are limited. A 2001 study conducted on patients with low- and high-grade gliomas found a

significantly higher rate of H63D allele (mostly in the form of heterozygotes) in high-grade gliomas compared to controls ($n = 203$).[88] This study offers the suggestion that further work is warranted.

Hepatocellular Carcinoma

Because liver dysfunction is readily seen in hemochromatosis, HCC was the first cancer to be linked to HFE polymorphism. Therefore, the literature is extensive compared to other cancers and strongly suggests that the C282Y variant increases the risk for HCC. An early study found that C282Y homozygosity was more common than expected in HCC, although the numbers were low and the penetrance of HCC as a phenotype related to C282Y homozygous genotype was low ($n = 3600$).[89] Later, a study conducted in Italy on 12 HCC patients and 130 controls did not find an increased prevalence of HFE polymorphisms in HCC, although 12 patients is too few to detect the effects that other groups reported.[90] A study of liver cancer and cirrhosis patients showed that C282Y homozygous genotype occurred significantly more often in both conditions compared to healthy controls. They did not see any differences in the frequency of HFE variant heterozygous genotypes ($n = 34$ liver cancer, 190 cirrhosis).[91] An Italian study conducted in 2001 found an increase in C282Y in HCC patients compared to healthy controls ($P < 0.03$) and found trends toward an association between C282Y and hepatitis virus markers and alcohol exposure, suggesting that C282Y could be interacting with these environmental factors to increase HCC risk ($n = 81$).[92] A 2002 study of patients with alcoholic and viral cirrhosis showed that C282Y polymorphisms were significantly more common in alcoholic cirrhosis patients with HCC compared to alcoholic cirrhosis patients without HCC. This effect was not seen in viral cirrhosis. In addition, all the H63D homozygotes in their study had cirrhosis, which points to a protective effect for H63D heterozygosity in alcoholic cirrhosis patients ($n = 179$ alcoholic cirrhosis patients, 98 viral cirrhosis patients, and 159 blood donor controls).[93] In a Swedish study, patients with HH had a 20-fold increase in liver cancer rates compared to expected population prevalence, but no other increase in GI cancers. The same study also saw nothing of note in first-degree relatives, but they did not perform genotyping and their study did not delve directly into iron levels ($n = 1847$ patients, 5973 first-degree relative controls).[94] A large multicenter study conducted in 2003 found no differences in HFE-variant allele frequency between patients with cirrhosis and HCC and patients with cirrhosis without HCC ($n = 133$ patients, 100 cirrhosis controls).[95] Another 2003 study found that C282Y heterozygosity was significantly more common in HCC cases than in control individuals and that C282Y heterozygosity in HCC was associated with higher levels of hepatic iron, serum ferritin, and transferrin saturation compared to WT HCC patients ($n = 137$ HCC, 107 cirrhosis, and 126 healthy).[96] This study suggests for some level of penetrance at the heterozygote genotype. A 2005 British study found that C282Y was associated with an increased risk of HCC (OR 14, 95% CI 5–37). They did not examine H63D ($n = 144$).[97] A 2007 Spanish study found that H63D was associated with risk of HCC in cirrhotic patients, while no significant difference in risk was seen for C282Y. For H63D, the OR of carriers versus wild type was 2.03 (95% CI 1.25–3.28; $n = 196$, 181 controls).[98] A 2008 study of cirrhotic patients showed that C282Y polymorphism and iron load were associated with a risk of HCC in alcoholic cirrhotic patients but not in HCV-related cirrhotic patients ($n = 301$).[99] A meta-analysis showed that, at least in cases with alcoholic liver cirrhosis as controls, the C282Y polymorphism was associated with HCC ($n = 1102$ HCC cases, 3766 controls).[100] In Egyptian HCC cases, both heterozygotes and homozygotes for H63D were more common in an HCC group than in matched controls, suggesting an association between the H63D allele and HCC in the context of cirrhosis and hepatitis C infection ($n = 200$ patients, 100 controls).[66] A 2011 study of Polish cirrhosis patients and controls did not find a significant association between HFE variant alleles and HCC. However, their study was fairly small (61 cirrhosis patients and 42 controls, with only 36 of the patients biopsied for liver iron stores), so it is possible that they were not sufficiently powered to detect subtler effects.[101] Although the evidence of effect is not universal, and the specific interactions with alcohol and hepatitis are not completely clear, there is sufficient evidence at present to safely claim that HFE variants are a risk factor for HCC. At present, it is not known whether these risk factors are due to iron overload alone or an off-target effect of HFE, and this question is best answered outside of the clinical population because it is ethically imperative to manage iron overload in known hemochromatosis patients. Furthermore, if untreated or late-treated populations of hemochromatosis patients could be identified, it would be useful to compare their oncologic epidemiologic data to a more aggressively treated set.

Prostate Cancer

The only available study on prostate cancer and HFE found no statistically significant difference in frequency of H63D or C282Y in 116 male breast cancer and 843 prostate cancer cases compared to 480 blood donor controls.[102] This study took place in Finland and used blood donors as controls, so more general population–based studies and a more representative control group are required before drawing a conclusion on prostate cancer.

Lung Cancer

As with several of the extrahepatic cancers, only one study has been published examining the association between lung cancer and HFE. A 2005 study of NSCLC found no evidence that these cancers select for HFE polymorphisms. The few cases of C282Y they found were present in both the tumor tissue and the normal surrounding lung ($n = 36$).[103] This piece of evidence alone says little about the effect of a host HFE

polymorphism on cancer, because it was designed to address the phenomenon of clonal selection by the tumor itself. Thus, at present, it is difficult to say whether lung cancer is among the cancers influenced by HFE status.

Ovarian Cancer

The only study exploring ovarian cancer and HFE variants found that C282Y polymorphism increased both the risk and aggressiveness of ovarian cancer in clinical populations ($n = 677$).[104] This finding demands follow-up, and more work is necessary before the repeatability and generalizability of these results are evident.

Conclusion

There exists a considerable body of evidence studying HFE in human populations, and the results are mostly inconsistent between studies. Given the prevalence of the HFE gene variants in the population and the ranges in dietary iron among different populations, the exact nature of the relationship of HFE polymorphisms to cancer is tremendously important but will be difficult to establish in population-based studies. Perhaps the most apparent shortcoming in the present literature is the overwhelming focus on risk. To fully understand the effect of HFE on tumor progression, we must also investigate the extent to which HFE modifies treatment effects and prognosis in the various cancers. In addition, we must consider whether variant HFE interacts with premalignant phenomena such as viral infection to affect tumor initiation. The findings in cervical cancer and virally mediated HCC support this hypothesis. The cell culture data suggest the existence of such effects, and how these findings translate to a clinically relevant effect requires further study.

In addition, to better understand the mechanistic significance of HFE polymorphisms in tumor progression, more controllable experimental systems are needed. One important factor that is sure to influence clinical studies from region to region is the association of HFE with ethnicity. This association introduces potentially confounding variables due to the links between ethnicity, socioeconomic status, gender (including menstruating versus nonmenstruating women), and various culturally influenced environmental exposures that need to be controlled in order to derive meaningful conclusions from this work. Importantly, an animal model would be free from these considerations, offering a means to directly interrogate mechanistic questions.

List of Abbreviations:

ALL: Acute lymphocytic leukemia
AML: Acute myelocytic leukemia
β2m: β2 microglobulin
C282Y: Cysteine-282-Tyrosine
Caco-2: A human colonic adenocarcinoma cell line
CD4: Cluster of differentiation 4 – Designates a protein on T-cells
CD8: Cluster of differentiation 8 – Designates a protein on T-cells
CDK1: Cyclin-dependent kinase 1
CNS: Central nervous system
CSF: Cerebrospinal fluid
DFO: Deferoxamine
EMT: Epithelial to mesenchymal transition
EPIC: European Prospective Investigation into Cancer and Nutrition
H63D: Histidine-63-aspartic acid
HCC: Hepatocellular carcinoma
HFE: high iron gene and its protein product
HH: Hereditary hemochromatosis
HPV: Human papilloma virus
ICAM-1: Intracellular adhesion molecule one
LCN2: Lipocalin 2
MAL-12: Murine hepatocyte cell line
MHC1: Major histocompatibility complex 1
N-Myc: DNA-binding transcription factor
NDRG1: N-myc downregulated gene 1
NSAID: Nonsteroidal anti-inflammatory drug
OR: Odds ratio
P16INK4A: Cyclin-dependent kinase inhibitor 2A
P21CIP1/WAF1: Cyclin-dependent kinase inhibitor 1
P27KIP1: A cyclin-dependent kinase inhibitor
P53: Tumor suppressor gene
ROS: Reactive oxygen species
RR: Relative risk
SW480: A human adenocarcinoma cell line
TFR1: Transferrin receptor 1
TGF-β1: Transforming growth factor β 1
UPR: Unfolded protein response

Author Contributions

Conceived the concepts: CLW, JRC. Analyzed the data: CLW. Wrote the first draft of the manuscript: CLW. Contributed to the writing of the manuscript: CLW, JRC. Agree with manuscript results and conclusions: CLW, JRC. Jointly developed the structure and arguments for the paper: CLW, JRC. Made critical revisions and approved final version: CLW, JRC. Both authors reviewed and approved of the final manuscript.

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