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HNF4α: a new biomarker in colon cancer?

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Colorectal cancer (CRC) is the fourth leading cause of cancer-related mortality worldwide with 50,000 deaths related to CRC annually in the USA alone. Early detection (Duke's stage A) predicts good survival (>90%) among CRC patients while the involvement of lymph nodes (stage C) or metastases (stage D) generally predicts survival below 50 and 10%, respectively. This highlights the need to identify prognostic biomarkers of early stage CRC as well as the need for more effective treatments at later stages. Here, we discuss the growing interest of HNF4α in CRC, as well as its potential as a biomarker for prognosis, susceptibility and treatment of this deadly disease.

HNF4α is a highly conserved member of the nuclear receptor superfamily of ligand-dependent transcription factors. It is expressed in the liver, pancreas, kidney, stomach, small intestine and colon, where it regulates many important aspects of epithelial cell morphogenesis and function. Nine different isoforms of HNF4α produced by alternate promoter usage and splicing have been identified thus far. Isoforms HNF4α1–3 are derived from the P1 promoter (P1-HNF4α), whereas HNF4α7–12 are derived from the P2 promoter (P2-HNF4α). The P1- and P2-driven proteins differ by approximately 16–29 amino acids in their N-terminal domain, which encodes an important activation function [101].

HNF4α is best known for its role in the liver and pancreas, including an inherited form of Type 2 diabetes referred to as MODY1, although it is increasingly being linked to liver and colon cancer [1–7]. Expression of P1- and P2-driven HNF4α in adult and embryonic liver, respectively, is essential for hepatocyte function and liver development [101]. Not surprisingly, loss of HNF4α leads to dedifferentiation of hepatocytes; it also leads to a switch from slow-growing to rapidly proliferating aggressive hepatocellular carcinoma (HCC) [1]. Recently HNF4α was identified as a central player in an inflammatory feedback loop involving IL-6 and miRNAs that leads to a decrease in *HNF4A* expression and an

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increase in HCC occurence [2]. While such reports do not distinguish between the P1- and P2-HNF4α isoforms, other studies have shown that P1-HNF4α expression is reduced in human and mouse HCC tissues while P2-HNF4α expression is aberrantly activated [6,8]. Taken together, these findings suggest that P1-HNF4α acts as a tumor suppressor in hepatocytes. However, the precise role of P2-HNF4α in HCC, and other cancers, remains to be determined.

Role of HNF4α in colon cancer

Several groups have used intestine-specific knockouts of *HNF4A* in the adult mouse and knockdown in human colon cancer cell lines to show that HNF4α is critical for the maintenance of epithelial cell function and normal colon physiology via regulation of the balance between proliferation and differentiation, immune function, ion transport, epithelial barrier function and oxidative stress [9–12]. Indeed, HNF4α appears to play a protective role against inflammatory bowel disease, an important risk factor for colorectal cancer (CRC); its expression is decreased in patients with Crohn's disease and ulcerative colitis [9]. A genome-wide association study also reported a potential association between a single nucleotide polymorphism in the 3′ UTR of *HNF4A* and susceptibility to inflammatory bowel disease [12]. However, there are contradictory reports on whether HNF4α acts as a tumor suppressor gene or an oncogene in colon cancer [4,13]. This could be due to the fact that these studies did not distinguish between the HNF4α isoforms: the intestine and colon are the only normal adult tissues that express both P1- and P2-HNF4α isoforms.

Development of monoclonal antibodies specific to the different HNF4α isoforms allowed Tanaka *et al.* to show that P1-, but not P2-HNF4α, is lost in colorectal carcinomas in humans [6]. In a study recently published in the *Proceedings of the National Academy of Sciences USA*, we followed up on that observation to decipher the mechanism responsible for the differential loss of HNF4α isoforms in CRC [3]. We demonstrated that P1-HNF4α protein, which is present only in the nucleus in normal tissue, was either lost or mislocalized to the cytoplasm in the majority (80%) of Duke's Stage C colon cancer patients, while P2-HNF4α expression was not reduced. We identified the probable mechanism for the differential loss of nuclear P1-HNF4α by showing that Src tyrosine kinase, a known player in CRC [14], phosphorylates Tyr14 in the HNF4α A/B domain. Interactions between the Src SH2 domain and phospho–Tyr14 subsequently result in the phosphorylation of two additional residues in the HNF4α ligand-binding domain. Since Tyr14 is present in the N-terminal region of P1- but not P2-HNF4α, the entire phosphorylation cascade is specific to P1-HNF4α. The end result is a decrease in protein stability and transcriptional activity of P1-HNF4α, but not P2-HNF4α. Finally, we demonstrate that loss of nuclear P1-HNF4α in human CRC samples correlates with staining for active Src [3].

HNF4α as a biomarker for colon cancer

Prognosis

Oshima *et al.* described a progressive loss of P1-HNF4α from Duke's stage A–D colon cancer and found that the lack of P1-HNF4α in stages C & D was associated with liver metastasis and poor prognosis [5]. By contrast, in our study we did not observe any correlation between P1-HNF4α status and the overall survival rate of CRC patients [3]. This could be attributed to the stage of the CRC patients in the Japanese study (combined stage C [n = 13; 20.6%] and stage D [n = 35; 55.6%]) versus our Australian cohort (stage C [n = 450; 100%]), and/or to genetic differences between the two cohorts.

While it is not yet clear whether the loss of P1-HNF4α alone can predict survival from CRC, it is possible that one of its target genes may serve as a biomarker for CRC. HNF4α

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has been shown to bind, and potentially regulate, approximately 11–22% of the genes in the HCC cell line Caco-2 [15,16]. One such gene is *GSTP1*. We recently reported that high GST-Pi expression is prognostic for poor outcome for stage C colon cancer patients: patients with a high nuclear accumulation of GST-Pi protein had a 5-year survival rate of 32% compared with a 53% survival rate for patients with low nuclear GST-Pi [17]. Interestingly, GST-Pi is also predictive for responsiveness to chemotherapy, although in a somewhat counter-intuitive fashion. The survival rate for patients treated with fluorouracil was higher in patients with tumors exhibiting high GST-Pi than those with low GST-Pi (as shown by [18]). An important consideration in the evaluation of both HNF4α and GST-Pi as potential biomarkers was the frequency of tumor cells staining positive for these proteins, as well as the subcellular distribution of the proteins within the tumor cells. It will be of interest in the future to ascertain whether the presence of distinct HNF4α isoforms in CRC tumors is related to the presence of GST-Pi or other biomarkers.

Susceptibility

In our study, we also found that single nucleotide polymorphism variants in HNF4α (L280F, rs6093980; P421L, rs6031602 and P436S, rs1063239) are differentially modulated by Src kinase [3]. Using cell-based assays we demonstrated that the variants had increased phosphorylation and decreased protein stability as well as transcriptional activity compared with wild-type HNF4α. These results suggest that an individual with any one of these single nucleotide polymorphism variants may be more susceptible to Src-mediated colon cancer, although this remains to be formally proven.

Drug treatment efficacy

The Src inhibitors dasatinib, AZD-0530 and SKI-606 are in Phase I and Phase II clinical trials for treatment of CRC. Currently, autophosphorylation of Src (pTyr419) and phosphorylation of its substrate paxillin (pTyr118) are used as potential biomarkers for assessing the efficiency of dasatinib treatment at inhibiting Src activity and tumor growth [19]. Another marker could be phosphoY14 of P1-HNF4α. It will also be of interest to determine whether expression and nuclear localization of P1-HNF4α in the colon is restored in the patients treated with the Src inhibitors. If it is found that loss of nuclear P1-HNF4α plays a causal role in CRC progression, the ability of a Src inhibitor to restore P1-HNF4α may prove critical for predicting the efficacy of these treatments.

HNF4α as a potential drug target for colon cancer

Nuclear receptors are major drug targets in several human diseases, including metabolic disorders and cancer. This is due, in a large part, to their hydrophobic ligand-binding pockets, which are natural targets for small molecules and which help regulate the recruitment of coregulators. Recently, we showed that in mammalian cell culture, as well as in the mouse liver, HNF4α binds in a reversible fashion to the essential fatty acid linoleic acid [20]. This finding opens up the possibility of HNF4α as a potential drug target. An HNF4α ligand/drug could potentially modulate its transcriptional activity or its level of expression. It could also possibly protect it from phosphorylation by Src.

In conclusion, HNF4α, long known as an important regulator of the differentiated state, has now been firmly linked to cancer. Any number of the many HNF4α target genes, in addition to HNF4α itself, could be a potential biomarker. Furthermore, the increasing genetic links between HNF4α and colon cancer could be used to predict susceptibility to CRC. Finally, we predict that any treatment (Src inhibitor, drug or diet) that increases nuclear P1-HNF4α protein levels may help slow colon cancer progression. While many questions remain to be addressed regarding the role of the different HNF4α isoforms in cancer, it is clear that HNF4α is a new target that warrants further investigation.

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