Epigenetic regulation of hypoxia-inducible factor -3α (HIF-3α) expression in colorectal cancer

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Immense proliferation of tumor cells leads to formation of hypoxic regions in many solid tumors, including CRC. Cells adjust to this microenvironment through hypoxia-inducible factor-α (HIF-α), which is an oxygen-sensitive component of the HIF transcription factor. Among three isoforms of HIF-α subunit: HIF-1α and HIF-2α share similar protein structure and together with constitutively expressed HIF-β subunit may activate HIF-dependent gene transcription. In contrast, HIF-3α act as a weak transcription factor and is reported to suppress HIF-1α or HIF-2α-mediated gene expression. Relatively little is known about regulation of HIF-3α gene expression in CRC. Since DNA hyper or hypomethylation of promoter regions alter expression of cancer related genes in CRC, presence of CpG island within promoter region of HIF-3α prompted us to investigate it role in colorectal tumorigenesis. Using real time PCR and western blotting we found significantly lower HIF3-α mRNA and protein level in primary colorectal tissue than in histopathologically unchanged colorectal tissue from the same one hundred twenty patients. Moreover, Kaplan-Meier analysis revealed a benefit of high HIF-3α protein level in cancerous tissue in patient survival compare to low HIF-3α protein level. The reduced HIF3-α expression was also correlated with increased DNA methylation in the CpG island of the HIF3-a assessed by bisulfite sequencing and high-resolution melting analysis (HRMA). Additionally we observed that 5-dAzaC significantly increased HIF3-α expression level in HCT116 cancer cells cultured in hypoxic conditions, whereas in normoxic neither DNA methylation nor changes in expression level were observed. Our findings present that HIF-3α is decreased in CRC and high HIF-3α protein level in cancerous tissue might be prognostic factor for CRC. Additionally, hypoxia-induced DNA hypermethylation of HIF3-α is very interesting observation of gene expression control.

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