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REPORT

Clinico-pathological significance of TNF alpha-induced protein3 (TNFAIP3) in Middle Eastern colorectal carcinoma

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A20, also known as TNF-alpha-induced protein3 (TNFAIP3), is a well-known negative regulator of the NF-kB activation pathway (Coornaert et al. 2009; Stilo et al. 2008) and functions as a tumor suppressor gene in lymphomas (Honma et al. 2009; Kato et al. 2009). Aberrant NF-kB regulation in colorectal carcinoma (CRC) is associated with poor prognosis and resistance to therapy (Horst et al. 2009; Kojima et al. 2004; Rakitina et al. 2003; Scartozzi et al. 2007). The current study deals with an interesting and novel topic about oncogenic role of inactivation of tumor suppressor gene A20 in colorectal cancer, the mechanism of A20 dysfunction, and its potential as a novel prognostic marker and therapeutic target. This is the first report of A20 alterations in colon cancer where we comprehensively investigated the genomic profile and epigenetic changes in A20 gene.

A better understanding of NF-kB modulators could lead to development of effective therapeutic strategies to target activated NF-kB (Rakitina et al. 2003). Mechanistically, A20 exerts its NF-kB inhibitory role in TNF signaling by the cooperative and dual-functioning actions of the deubiquitinating and ubiquitinating enzyme activities located at the N and C terminus, respectively, of the A20 protein (Coornaert et al. 2009). The amino terminal of A20 protein containing the deubiquitinating enzyme targets removal of ubiquitin moieties from receptor-interacting protein (RIP1), an essential mediator of the proximal tumor necrosis factor receptor signaling complex in NF-kB activation (Wertz et al. 2004). This is followed by polyubiquitination of RIP by the ubiquitin ligase located at the carboxy terminal of A20 protein, thereby targeting RIP for proteosomal degradation. Recent studies have shown that A20 functions as a tumor suppressor gene in several types of lymphoma wherein not only is A20 inactivated by promoter methylation (Chanudet et al. 2009), but it is also subject to gene deletion and mutation. Although A20 has been reported as a novel tumor suppressor gene in NHLs, the role of A20 in colorectal carcinoma is not yet known. In this study, we screened for *A20* gene copy number changes and A20 expression in a large tissue microarray cohort of 434 colorectal carcinomas and adenoma samples. We further investigated the incidence of *A20 mutations* and *A20 promoter methylation* and the relationship of A20 alterations with CRC progression and phenotype.

The incidence of A20 mutations, A20 deletions, A20 promoter hyper-methylation was 2.5%, 15.3%, and 50.8%, respectively. We analyzed the entire coding region of A20 gene in 116 CRC samples and 14 CRC cell lines and found that only three CRC samples and two CRC cell lines (LOVO and HCT-15) were mutated. Most of the *A20 mutations* found in Hodgkin's and mediastinal lymphomas were nonsense or frameshift mutations that prevented production of full-length A20 protein (Chanudet et al. 2009; Honma et al. 2009; Schmitz et al. 2009). The three A20 mutations found in our study were replacement mutations. Two of these three mutations in our study were localized in the zinc finger (ZF) domains at the C terminus

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of the protein. The A20 ZF domain functions as an E3 ubiquitin ligase, adding K48-linked polyubiquitin chains to RIP1 and targeting the protein for proteasomal degradation. Most of the mutations seen in the three CRC samples as well as the two cell lines were missense mutations (four out of five). A20 deletions were seen in 15.3% (65/424) of the CRC samples analyzed by FISH. Homozygous deletions, i. e., presence of two centromeric signals but no A20 signals, were seen in four of the 65 (3.1%) deleted cases. Although A20 mutations were not observed in the CRC with A20 deletions, six cases showed co-existence of A20 methylation and A20 deletions. Thus, A20 alterations are widely prevalent in colorectal carcinoma, and A20 promoter methylation leads to reduced expression of A20 protein.

A20 promoter hyper-methylation was correlated with loss of protein expression and associated with histology subtype of adenocarcinomas differentiated tumors and CRC located on the left side. A20 protein expression was significantly higher in colorectal adenomas as compared to colorectal carcinomas. A20 expression identifies a subgroup of colorectal cancers which show a poor outcome across all subgroups, in stage III alone, stage II and III subgroup, and also stage III and IV subgroup. Reduced A20 expression was an independent prognostic marker in colorectal carcinoma. Together, our results establish A20 as a key tumor suppressor molecule in colorectal carcinogenesis and identify a subgroup of CRC patients with reduced A20 expression showing a poor overall survival. *A20 promoter methylation* is the key epigenetic mechanism leading to suppression of A20 expression. Interestingly, complete loss of A20 expression results from multiple hits like A20 deletion and or mutation with methylation in accordance with Knudson's two-hit hypothesis. Our findings indicate that A20 is a tumor suppressor in CRC that can be used as a potential prognostic biomarker.

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