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Letter to the Editor

Frameshift mutations in *Fas*, *Apaf-1*, and *Bcl-10* in gastro-intestinal cancer of the microsatellite mutator phenotype

Dear Editor,

In a letter to the editor in a recent issue of Cell Death and Differentiation, Abdel-Rahman *et al*[1]. reported the absence of mutations in the death pathways gene *Fas* (*Apo-1/CD95*) in colorectal carcinomas. From the absence of mutations in 24 colon cancers, 12 of which were classified as replication error positive (RER+), Abdel-Rahman *et al*[1]. concluded that such mutations confer no substantial growth advantage in colorectal carcinogenesis. In agreement with this report, we identified *Fas* mutations in only 10% of colon and gastric cancers of the microsatellite mutator phenotype (MMP), also denominated as RER or microsatellite instability (MSI). Mutations were also found in *Apaf-1* and *Bcl-10*, two other genes involved in the cell death pathways. The mutations were detected in mononucleotide tracts within these three genes (Figure 1). The frequency of these frameshift mutations was low (Table 1) and they appeared to be heterozygous (Figure 2). However, considering the peculiar features of these tumors, we suggest that these frameshift mutations contribute to cancer progression by providing survival advantage.

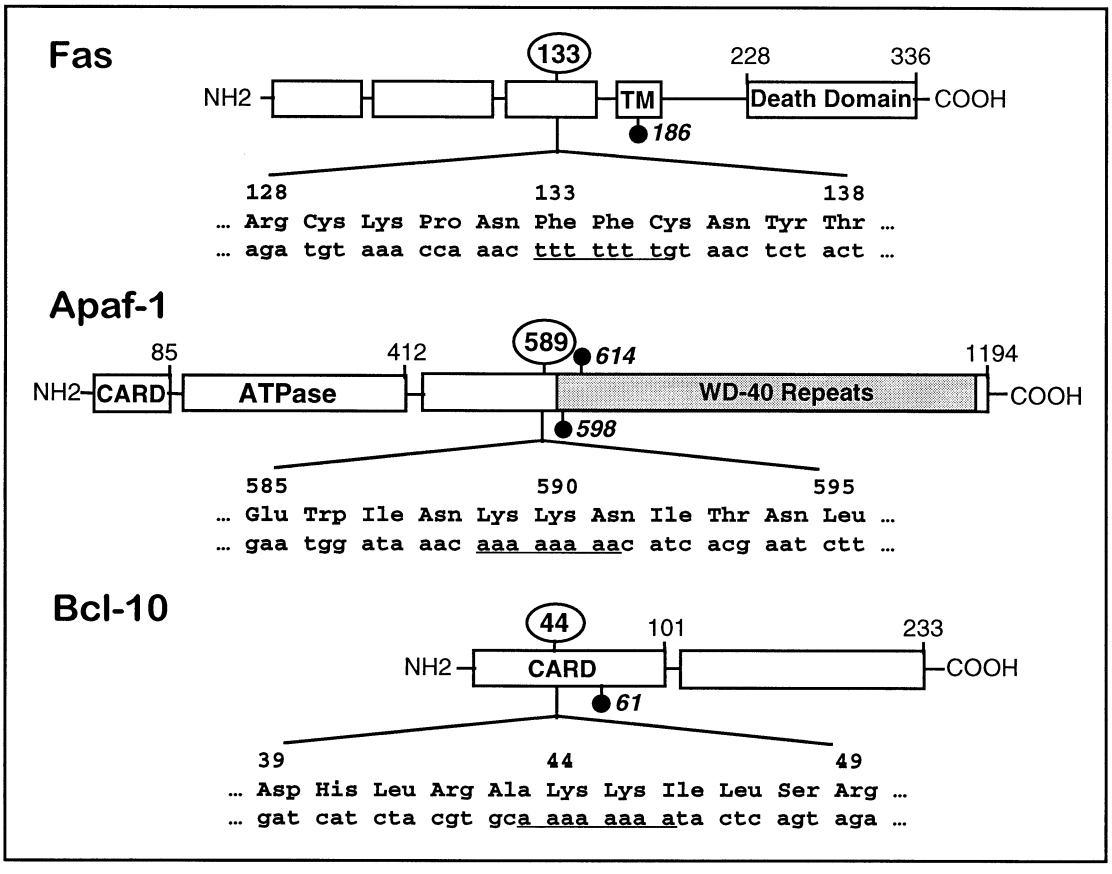

Figure 1 Position of mononucleotide tracts in pro-apoptotic genes *Fas*, *Apaf-1* and *Bcl10*. Frameshift mutations at these positions are likely to inactivate protein function because the resulting truncations eliminate functional domains for apoptosis in each of these three genes.[7–9] FAS plays an apoptotic role in response to *c-Myc*, DNA damage and T-cell mediated cytotocixity, through the caspase cascade.[7,10] Apoptotic protease activating factor-1 (APAF-1) is a proximal activator of caspase-9 in cell death pathways that trigger mitochondrial damage and cytochrome *c* release.[8,11] *Bcl10* is involved in the execution of apoptosis.[9,12,13] No mutations have been described in *Apaf-1* in human cancer. A low frequency of missense mutations in *Fas* has been described in several human malignancies.[14–16] Mutations in *Bcl10* have been found in several cancers, including colon cancer cell lines.[9,13,17] A similar truncated form of Apaf-1 (codons 1–570) fails to induce apoptosis and to activate pro-caspase-3.[18] Although frameshift mutations in these genes could have a negative dominant effect since these proteins oligomerize, we favor an alternative accumulative haploinsufficiency hypothesis (see text). TM: Transmembrane domain; CARD: Caspase recruiting domain. Positions of stop codons after frameshifts are indicated in italics by dots (+1 and −1 bp, above and below, respectively). Not to scale

Table 1 Frameshift mutations in 30 colon and 20 gastric MMP+ cancers were analyzed by PCR amplification of the coding regions flanking the mononucleotide tracts present in these genes (Figure 1)

	Colon	Gastric
Fas	3 (10%)	2 (10%)
Apaf-1	4 (13%)	3 (15%)
Bcl-10	4 (13%)	2 (10%)

Frameshift mutations in these three apoptotic genes were absent in 60 colon cancers without the MMP. Even in the presence of the MMP, the mutation rates in these short repeated sequences appear to be below detectable limits in the absence of a positive selection, because no mutations are found in many repeated sequences of identical length in noncoding regions nor in other genes not implicated in cancer[6,19,20]

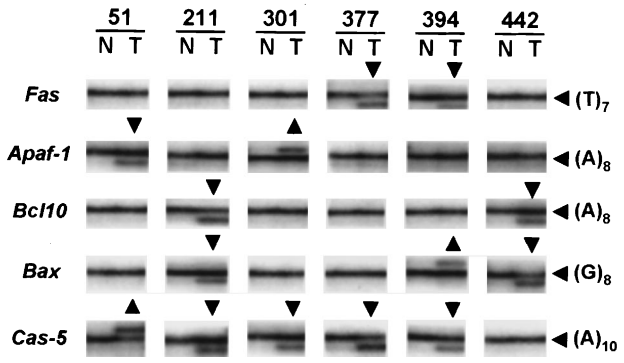

Figure 2 Frameshift mutations in apoptotic genes in colon tumors of the microsatellite mutator phenotype. The position and size of the wild-type PCR product are indicated at right, and triangles pointing up or down indicate insertions or deletions of one nucleotide. T and N: tumor and adjacent normal colon tissues. Frameshift mutations in Bax and Caspase 5 have been previously described.[6,19,20] No other caspases were analyzed for mutations because they do not have mononucleotide tracts in the coding regions of sufficient length (7 or more bp). The only exception is Caspase 1, which has a $(A)_8$ tract, but no mutations were found.[19] Amplification of *Bax* and *caspase-5* and analysis by polyacrylamide gel electrophoresis and autoradiography, was as described.[6,19,20] Amplification of *Fas*, *Apaf-1* and *Bcl10* was done with up and down PCR primers: 5′ ACC CAG AAT ACC AAG TGC AG 3′ and 5′ TGC AAG GGT CAC AGT GTT CA 3′ for *Fas*; 5′ GTT ACT TTT TTC CCT GTA TTT AGA AAC 3′ and 5′ TAT TCT CTG ACC ATC CTC AG 3′ for *Apaf-1*; and 5′ CAT AGC TGA GAG ACA TTT TG 3′ and 5′ AGC CCT TTT TCT ACT TGA TG 3′ for *Bcl10*

The MMP pathway for gastrointestinal cancer presents two paradoxical features at first sight. First, despite accumulating hundreds of thousands of clonal somatic mutations in simple repeated sequences, these tumors exhibit a low mutation incidence in *APC*, *K-ras* and *p53*[2] prototypical cancer genes for colorectal carcinogenesis.[3] Second, while ubiquitous mutations in non-functional poly (A)n sequences (such as the poly A tails of the *Alu* repeats), are biallelic,[2] these tumors also accumulate many monoallelic (i.e. heterozygous) mutations in functional sequences, such as the coding regions of mutator (*hMSH3*, *hMSH6*),[4] suppressor (*p53*, *TGFβRII*)[2,5] and apoptotic (*Bax*)[6] genes. In contrast, the manifestation of the tumor phenotype by cancers without the MMP is usually associated to the biallelic mutational inactivation of a few cancer genes such as *APC* and *p53* tumor suppressors.[3]

The first paradox may be explained by the existence within some genes of simple repeats which are preferred targets for the MMP. Thus, in the presence of the mutator phenotype, mutations in these genes (i.e. *Bax*) occur sooner than in other genes of the same oncogenic (i.e. apoptotic) signaling pathways that do not have these repeats (i.e. *p53*).[6] We propose here a model to explain the second paradox. Due to the exacerbated mutator phenotype of these tumors, their ability for escaping apoptosis may be facilitated by the accumulation of heterozygous mutations in multiple genes whose products play partially redundant and partially synergistic roles at different points of the apoptotic signaling network. This accumulation of heterozygous mutations presumably reduce the homeostatic threshold amount of the corresponding pro-apoptotic gene products.

In agreement with this hypothesis, most MMP+ tumors had frameshift mutations in more than one of these genes (Figure 2). Due to the still limited knowledge of the human genome and the strong mutator phenotype of these tumors, it appears very unlikely that these are the only death pathway genes mutated in cancer of the MMP. This accumulative haploinsufficiency model is not restricted to apoptotic pathways, but also applies to other networks involved in the homeostatic control of genome integrity and cell proliferation.

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