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LETTER TO THE EDITOR

Dear Sir,

Resistance to CD95-mediated apoptosis in breast cancer is not due to somatic mutation of the CD95 gene

Resistance to CD95 (Apo-1/Fas)-mediated apoptosis is a typical feature of breast cancer cells. Recent studies identified deleterious mutations of the CD95 gene not only in a variety of B cell lymphomas but also in a number of solid tumor entities. Therefore, we amplified and sequenced selected regions of the CD95 gene from 48 breast cancer cases and 10 cell lines but no mutation was found. In the presence of both polymorphic alleles, loss of heterozygosity was excluded in 27 informative cases. We conclude, that relevant somatic mutations of the CD95 gene occur, if at all, at a low frequency and are not the primary cause for resistance to CD95-mediated apoptosis in breast cancer.

Key words: *apoptosis; breast cancer; somatic mutation; tumor suppressor gene; CD95*

CD95 (Apo-1/Fas) represents a major regulator of apoptosis and was recently identified as a tumor suppressor gene.[1] *Mutations within the germline of the CD95 gene are associated with an increased risk of B cell lymphoma and solid tumors.*[2] *Moreover, there is now broad evidence that lymphomas derived from (post) germinal center B cells frequently harbor somatic CD95 mutations, resulting in CD95 deficiency and resistance to CD95-mediated apoptosis*[3–5]

Recently, the group of NJ Yoo (Seoul, Korea) reported on the occurrence of somatic CD95 mutations in 4 solid tumor entities.[6–9] *The fraction of tumors carrying a mutated CD95 gene in bladder cancer, malignant melanoma, non-small cell lung cancer and squamous cell carcinoma ranged between 4 and 28%. Yoo and colleagues therefore proposed that silencing of CD95 function by destructive somatic mutations might represent a ubiquitous strategy of epithelial tumors conferring resistance,* e.g.*, towards CD95 ligand expressing cytotoxic T cells.*

Given that breast cancer cells in most if not all cases lose CD95 expression and sensitivity to CD95-mediated apoptosis during tumor progression,[10,11] *we analyzed selected regions of the CD95 gene in 48 cases of breast cancer and 10 breast cancer cell lines.*

MATERIAL AND METHODS

Two regions of the CD95 gene (Fig. 1) were amplified and sequenced from genomic DNA extracted from 48 microdissected breast cancer specimens and 10 breast cancer cell lines as previously described.[5] *Nine low grade (GI), 20 intermediate grade (GII) and 19 high grade (GIII) breast cancers (mostly invasive ductal carcinomas) were studied.*

RESULTS

In order to identify somatic mutations impairing CD95 mRNA expression, we amplified and sequenced 5′ regulatory regions including a p53-responsive intronic enhancer. Identifying 2 novel germline polymorphisms but no somatic mutations in this region, we amplified both polymorphic alleles from all 27 informative cases and cell lines, indicating that allelic loss of the CD95 locus occurs, if at al, at a very low frequency in breast cancer. In a search for loss-of-function mutations, we amplified and sequenced the last exon coding for the death

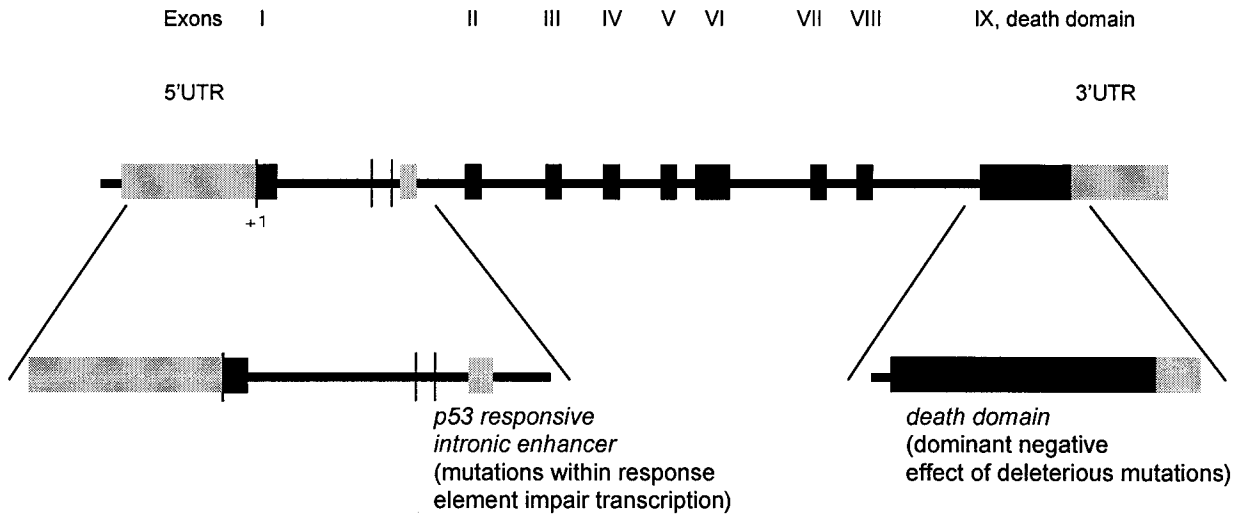

FIGURE 1 – Organization of the CD95 gene and PCR strategy. The organization of the CD95 gene (comprising exons I to IX) is depicted (not to scale). From breast cancer samples a 500 bp fragment encompassing 5′ untranslated (5′UTR; gray boxes) and coding (black boxes) regions of exon I and a p53 responsive intronic enhancer was amplified as previously described.[5] In addition, a 440 bp fragment containing exon IX coding for the death domain of CD95 was amplified. Two novel germline polymorphisms are depicted by vertical lines.

domain from 48 breast cancer cases and 10 cell lines. The death domain is required and sufficient for the transduction of the apoptosis-signal and was identified as a mutational hotspot in recent studies (reviewed in reference 1). Mutations in this region act in a dominant-negative way. Sequence analysis of the death domain, however, did not reveal any mutation in the breast cancer samples.

DISCUSSION

Inactivation of the CD95 gene by somatic mutations within exons II to VIII (Fig. 1; which may have been missed in this analysis) would require biallelic mutation or monoallelic mutation in conjunction with loss of heterozygosity. Allelic loss of the CD95 gene, however, was excluded for 27 informative cases and cell lines.

We conclude that loss of CD95 expression and function, which is a common feature of breast cancer, is, if at all, only in rare instances a result of somatic mutation. Based on the finding of truncating CD95 mutations in a substantial fraction of bladder cancer, non-small cell lung cancer, malignant melanoma and squamous cell carcinoma, NJ Yoo and colleagues proposed that somatic mutation of the CD95 gene may be a common mechanism for CD95-inactivation and acquisition of resistance to CD95-mediated apoptosis. Since recent studies reported on the absence of somatic CD95 mutations in colorectal carcinomas[12] and ovarian cancer,[13] and CD95 mutations were missing in the breast cancer samples studied here, this mechanism is apparently not ubiquitous.

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