MOLECULAR ANALYSIS
OF THE HUMAN
FAS GENE IN COLORECTAL CANCER

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by

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Summary

Fas is a cell surface receptor which, when bound to its endogenous ligand, transduces apoptotic cell death signals. Fas is normally expressed throughout the epithelial cells of the colon, but its expression is reduced or absent in almost 90% of colorectal carcinomas. Loss of Fas in the colon may contribute to the reduced apoptotic capacity of colorectal cancer cells. The primary aims of this thesis were to determine the molecular mechanism by which expression of Fas is lost in colorectal tumours; and to investigate the effects of re-introducing Fas into colon cancer cells.

The expression of Fas mRNA transcripts was analysed in both normal colonic mucosa and tumours of various grades. Normal mucosa expressed Fas mRNA constitutively, but the levels of Fas mRNA were reduced or absent in 17 out of 28 (60%) colonic tumours analysed. Expression of Fas mRNA was also low or absent in two out of five colon cancer cell lines. This indicates that the reduction in expression of Fas protein in colorectal tumours is mediated at the transcriptional level. Expression of the Fas ligand was also examined in colonic tissues by RT-PCR and was detected in all normal and tumour samples examined.

The human Fas gene was then analysed in colonic tumours for allelic loss, gross rearrangements and point mutations, as possible causes of reduced expression of Fas mRNA. Allelic loss of the Fas gene was detected by a polymerase chain reaction (PCR) -based restriction assay, utilising restriction enzyme polymorphisms in the Fas gene. Loss of heterozygosity at the Fas gene locus was detected in six of 38 informative colorectal tumours (16%), indicating that allelic
loss of *Fas* had occurred. Fifteen of the informative colonic tumours had also been analysed for expression of Fas mRNA, and 10 (67%) of the tumours showed reduced or no expression. Three tumours with allelic loss of the *Fas* gene did not express any detectable Fas mRNA, suggesting that the remaining allele had also been inactivated in these tumours.

Hybridisation of Southern blots with *Fas*-specific DNA probes detected no rearrangements or dosage changes of the *Fas* gene in 64 colonic tumours. Mutational analysis of the intracellular signal transduction domain (the "death domain") of *Fas*, using the PCR-single strand conformation polymorphism (SSCP) technique, failed to detect any point mutations in genomic DNA from 43 tumours, including the tumours with allelic loss of the *Fas* gene. Taken together, these results show that the only major structural defects of the *Fas* gene in colon cancer are the loss of an allele in a small percentage of tumours. This suggests that loss of expression of Fas in most colon carcinomas is not mediated at the genetic level.

Methylation of cytosine residues in the promoters of many genes can inhibit transcription of mRNA during tumorigenesis. The promoter region of the *Fas* gene was examined by Southern blotting for changes in methylation, in DNA isolated from 28 colonic tumours. No methylation of the promoter region was detected in either normal or tumour tissues. It is therefore likely that the loss of a transcription factor, or the presence of a repressor protein, inhibits transcription of the *Fas* gene in colorectal tumours.

The functional significance of reduced levels of Fas in colon cancer was investigated by transfection of the *Fas* gene into the Fas-negative colon cancer cell
lines LIM1215 and SW620. No stable clones of LIM1215 cells expressing the exogenous Fas gene could not be generated, while only two clones expressing Fas mRNA could be isolated from the SW620 cell line, suggesting that expression of Fas in colorectal cancer cells is incompatible with cell growth. These preliminary findings support the hypothesis that down-regulation of Fas is required for the survival of colorectal cancer cells.

In addition to the studies of colorectal cancer, an animal study was undertaken to investigate the role of Fas signalling in hormone-dependent involution of the prostate gland. This study examined the levels of Fas and Fas ligand mRNA in the prostate glands of rats castrated for various periods, however no upregulation of either Fas or Fas ligand mRNA was detected.