New Doctorial Cancer Research

DNA Methylation in Cancer Development: Lessons Learned from Tumors of the Testis and the Large Bowel

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Ph.D. Dissertation date: Dec. 15, 2005
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The current thesis investigates DNA methylation in cancer development by exploring this phenomenon in two cancer diseases, testicular cancer and colorectal cancer. This study shows that the two cancer types display different methylation profiles and identifies novel gene targets inactivated by DNA hypermethylation during the tumorigenesis in each of the two organs, as well as in their in vitro models.

In testicular germ cell tumors, CpG island promoter hypermethylation is associated with tumor histology. Nonseminomas display methylation frequencies of target genes comparable to those of other cancers, whereas seminomas in general are devoid of methylation. Among the various nonseminomatous histological subgroups, teratomas display more and embryonal carcinomas less methylation than do the remaining nonseminomatous subtypes. The methylation frequencies vary along the embryonic and extraembryonic differentiation lineages of the testis tumor model, mimicking the epigenetic reprogramming during early embryogenesis. Epigenetic target genes novel in testicular tumorigenesis are identified, including \( MGMT, SCGB3A1 \) (\( \text{HIN}-1 \)), and \( \text{HOXA9} \).

DNA hypermethylation is found in small benign lesions of the colorectum and typically increases in frequency as the tumor progresses into malignancy. The following genes, \( \text{ADAMTS1}, \text{CRABP1}, \text{HOX49}, \) and \( \text{NR3C1} \), are identified as novel epigenetically regulated target genes in colorectal tumorigenesis. Several associations were found among clinicopathological variables and gene methylation, which may aid in the future molecular assisted classification of colorectal cancer patients. Furthermore, colon cancer cell lines were found to be representative epigenetic models for the in vivo situation.

Comment by Per Guldberg

An Introduction part of the thesis presents the literature of the field of epigenetics in cancer in an excellent manner. The thesis includes six separate papers that summarize the experimental work. Overall, the candidate and her coauthors have used various technological approaches combining the detailed biology with large-scale microarray analyses. Indeed, this has resulted in several novel findings and provided new knowledge about the tumorigenesis of the testis and the large bowel, the two disease models used in this work. By combining in vitro studies with those of primary tumors, and using strict selection criteria, the number of expected false positives after treatment with aza-deoxy cytidine was
shown dramatically reduced. This then led to the identification of, among others, three novel genes in colorectal tumorigenesis. These genes all have interesting functions, and their potential involvement in other cancers remains to be seen.

FIGURE 1. Methodological approach for selecting candidate genes inactivated by hypermethylation. Methylation was removed in four colon cancer cell lines by culturing them in the presence of 5-aza-2'deoxycytidine. Subsequent gene expression in these and their untreated counterparts was analyzed by microarrays, and 1059 genes responded to the treatment. To increase the likelihood of selecting relevant genes from this list, and to avoid false positives, several strict criteria were applied. First, only genes that responded in at least three out of the four cell lines analyzed were considered candidates, reducing the list to 93 genes. Sixty of these 93 genes were down-regulated across a panel of colorectal carcinomas compared with normal mucosa. Among these 60 genes, 21 contained a CpG island in their promoter. Among the 21, four novel candidates encoding proteins with potential roles in tumor development were subjected to downstream analyses, whereupon three were shown to be inactivated by promoter hypermethylation in colorectal carcinomas and adenomas.