Department of Microbiology, Tumor and Cell Biology

Employing epigenetic marks to detect cancer. Studies on nasopharyngeal carcinoma and lung cancer

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i hörsal Atrium, Nobels väg 12B

Onsdag 25 Februari 2015, kl 09.30

av

Imran Nawaz

Huvudhandledare: Professor Ingemar Ernberg Karolinska Institutet Department of Microbiology, Tumor and Cell Biology

Bihandledare: Associate Professor Li-Fu Hu Karolinska Institutet Department of Microbiology, Tumor and Cell Biology

Fakultetsopponent: Doctor Andreas Lennartsson Karolinska Institutet Department of Biosciences and Nutrition

Betygsämnd: Associate Professor Charlotte Ling Skåne University Hospital Lund University Diabetes Center

Professor Boris Zhivotovsky Karolinska Institutet Institute of Environmental Medicine

Professor Laszlo Szekely Karolinska Institutet Department of Microbiology, Tumor and Cell Biology

Stockholm 2015
ABSTRACT

Tumor suppressor genes (TSGs) or oncogenes aberrantly methylated in transcription control regions during early carcinogenesis are potential tools for early detection of cancer. We have identified suitable genes and explored assays based on their methylation status aiming for early detection of cancer: nasopharyngeal carcinoma (NPC) and non-small cell lung cancer (NSCLC).

We established and developed further the “multiplex methylation specific-PCR (MMSP)” assay designed to detect the tumor-specific methylation status of several NPC-related genes (paper I). It provided information about the methylation status of multiple genes simultaneously in a single PCR with small amounts of tumor DNA derived from nasopharyngeal swabs. It was shown to be applicable with DNA from as few as 10 cells. The detection rate of NPC from nasopharyngeal swabs was 98%. The false positive rate was zero.

We employed the MMSP assay on NPC tumors from two other regions (Morocco and Italy) and compared our results with those on Chinese NPC patients from paper I (paper II). We also did a pilot study using sera from Italian and Chinese NPC. We updated the panel of MMSP markers and modified the assay to improve its applicability to NPC from different geographical locations. We could detect at least any one methylation marker gene in 97% of the EBNA1 positive samples with a specificity of 94%, while the results on sera were less informative than using swabs.

We used an established NotI microarray method to identify gene losses (by deletion or methylation) in chromosome 3 of NPC tumors (paper III). This chromosome is known to contain TSGs involved in many cancer types. Ten candidate TSGs were found. Among them, the CpG rich area in the promoter region of Integrin α9 (ITGA9) was confirmed to be hypermethylated in NPC by bisulfite cloned sequencing, bisulfite pyrosequencing and methylation specific PCR. ITGA9 was downregulated in NPC clinical samples and 5-aza-2′-deoxycytidine restored the expression of ITGA9 in NPC derived cell lines. The functional role of ITGA9 downregulation in NPC should be explored further.

We developed an MMSP assay for analysis of the methylation status of multiple potential TSGs in NSCLC samples (paper IV). Thirty-eight potential TSGs were selected, based on literature search, genome-wide CpG methylation and expression microarrays performed on NSCLC tissues and matched control tissues. After evaluation by methylation specific PCR (MSP) six of these genes were selected for inclusion into the MMSP assay. Subsequently, 70 NSCLC DNA samples with matched controls and 24 non-cancerous DNA samples were screened with this assay. With a cut off of methylation of at least any two of these marker genes 87% of the cancer samples were detected with a specificity of 94%. Early stage I or II NSCLC showed a 100% specificity and 86% sensitivity.