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A PRELIMINARY STUDY OF DIFFERENTIALLY EXPRESSED GENES IN MALAYSIAN COLORECTAL CARCINOMA CASES

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Abstrak: Setakat ini, mekanisme genetik yang lengkap tentang kemajuan adenoma kepada karsinoma dalam karsinoma usus besar masih belum diketahui. Demi memperoleh maklumat genetik tentang lintasan karsinogenesis ini, kami menggunakan kaedah pencarian gen yang diekspres secara berbeza dalam tumor karsinoma usus besar. Profil pengekspresan gen daripada kes karsinoma usus besar dikaji menggunakan sistem DNA Microarray. Kami melaporkan pengawalaturan tinggi dan rendah untuk 819 dan 98 gen masing-masing dalam tumor relatif kepada kawalan normal berkenaan. Corak pengekspresan berbeza dalam 121 gen adalah kekal dalam semua tumor yang dikaji. Tiga puluh tiga daripada gen-gen ini adalah gen protein ribosom (RP). Perbandingan data dengan pangkalan data pengekspresan gen domain awam (*Cancer Gene Expression Database, CGED*) menunjukkan 47 gen pengekspresan berbeza adalah konsisten. Dua puluh dua daripada gen-gen ini adalah gen RP. Antara semua gen RP yang dikenal pasti dalam kajian ini, corak pengekspresan untuk enam gen adalah selaras dengan sorotan kajian. Pengekspresan tinggi gen RP *L32* dilaporkan buat pertama kali dalam kajian ini dan telah disahkan melalui penganalisaan *RT-PCR*. Gen-gen yang tidak berkaitan dengan RP tetapi penting untuk dipertimbang adalah gen *tumour susceptibility* (*TSG101*) dan *20-kDa myosin light chain* (*MLC-2*). Walaupun penganalisaan *microarray* kami berdasarkan saiz sampel kecil (n = 2), kajian awal ini mengenal pasti banyak gen yang dikaitkan dengan konteks pembentukan dan kemajuan karsinoma usus besar buat pertama kali. Maka, penemuan kami membekalkan maklumat baru untuk kejadian genetik dalam proses karsinoma usus besar.

Abstract: Presently, the complete genetic mechanisms for the progression of adenoma to carcinoma for colorectal carcinoma (CRC) remain largely unclear. In order to obtain genetic information of this cancer pathway, we searched for differentially expressed genes in tumours of CRC. Gene expression profiles from CRC cases were assessed via the DNA microarray system. We report up-regulation and down-regulation of 819 and 98 genes respectively, in the tumours relative to their normal controls. The differential expression patterns of 121 genes were persistent in all tumours. Thirty three of these are ribosomal proteins (RPs) genes. Comparison of the 121 genes with a public domain gene expression database, the Cancer Gene Expression Database (CGEP), revealed 47 genes to be consistently differentially expressed in colorectal tumours. Among these, 22 are RP genes. Among all RP genes identified in this study the over-expression pattern for six of them is consistent with literature. The up-regulation of RP *L32* in CRC tumours was demonstrated for the first time in this study and it was also verified via reverse transcription-polymerase

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chain reaction (RT-PCR) analysis. Non-RP genes worth noting are the tumour susceptibility gene (*TSG101*) and the 20-kDa myosin light chain (*MLC-2*). Albeit small sample size (n = 2 for microarray analysis), our preliminary studies revealed many genes that are brought into the context of CRC tumourigenesis for the first time, thus providing new clues to the genetic events during colorectal carcinogenesis.

Keywords: Colorectal Carcinoma, DNA Microarray, Ribosomal Proteins (RP), *TSG101*, *MLC-2*.

INTRODUCTION

Colorectal carcinoma (CRC) is one of the human cancer models where the progressive histopathological stages correlate with gradual but sequential perturbation of specific genes. These basic and sequential events that correlate with the adenoma-carcinoma progression have been reviewed by Fodde *et al.* (2001). Generally, in the case of a type of inheritable CRC – the familial adenomatous polyposis (FAP), initiation of tumour formation and clonal evolution of tumourigenic cells can be triggered by germline inactivating mutations in the adenomatous polyposis coli (*APC*) gene. In the context of Wnt-signaling pathway, the improper function of mutant *APC* leads to the stabilization of *β-catenin*, and hence the formation of the *β-catenin/TCF-LEF* (T-cell factor-lymphoid enchancer factor) complex. This results in the ectopic activation of oncogenes, one of which is *k-ras*. This in turn leads to a proportion of precancerous colorectal cells becoming adenomatous polyps. Further genetic disruption specifically in the form of Loss of Heterozygosity (LOH) at chromosomes 18q and 17p involving *SMAD2/SMAD4* and *TP53* genes respectively, causes the malignant transformation of benign adenomas to invasive carcinoma of the colorectum. This basic sequence of genetic events in colorectal cancer evolution may also be true for sporadic CRC, as *APC* mutations have been verified in early stages of sporadic CRC tumours (Powell *et al.* 1992). Contrary to the phenomenon of *APC*-initiated tumourigenesis, genetic defects (causing tumour progression) in the second familial forms of CRC, the Hereditary Nonpolyposis Colorectal Cancer (HNPCC), occur in the DNA mismatch repair (MMR) genes (Kinzler & Vogelstein 1996). In the HNPCC cases, MMR deficiencies concomitantly cause adenomas to acquire higher rate of mutation relative to normal colorectal cells. The resultant accumulation of mutations in oncogenes and tumor suppressor genes will lead to malignant transformation of adenomas (Kinzler & Vogelstein 1996).

The variety of genetic pathways that pertain to the adenoma-carcinoma sequence between FAP (and some sporadic CRC) and HNPCC reflects the complexities of the molecular mechanisms underlying tumor initiation to malignant progression of colorectal carcinoma. Presently, the understanding of these mechanisms remains basic if not partially elucidated. Of late, several groups have attempted to study the molecular events of colorectal carcinoma using approaches of gene expression analysis. These include the Serial Analysis of Gene Expression (SAGE) analysis (Zhang *et al.* 1997), the Affymetrix Human GeneChip™ (6500 and 6800) Set oligonucleotide arrays (Notterman *et al.* 2001), the 19,200-Element Complementary DNA microarray (Hedge *et al.* 2001), the