Anchorage independence in tumor cells: Genomic structure and functional role of the *Cdkn2a/2b* tumor suppressor region in the rat

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ABSTRACT

The two most significant characteristics of tumor cell growth *in vitro* are anchorage independence and immortality. The ability to form colonies in soft agar (anchorage independent growth) has been instrumental as an *in vitro* indicator of late stage tumor cells with metastatic potential. However, the mechanisms regulating the anchorage dependent growth are still not well understood.

With particular reference to anchorage independence, the present work is focused on a region on rat chromosome (RNO) 5q31-q35 involving the *Ifna-Cdkn2a/2b-Mtap* loci. In a previous study using a mouse hepatoma-rat tumor hybrid system it was observed that deletion of this region functionally correlates to anchorage independence. In this thesis, a stepwise analysis has been applied to characterise the region, both at the genomic and functional levels.

Firstly, at the genomic level, nine new DNA markers were derived from the deletion region on RNO5 using the RDA method. These markers contributed to the construction of (a) a FISH framework map for RNO5q12-q36 region and, (b) YAC contigs of the *Ifna-Cdkn2a/2b-Mtap* region and a PAC contig of the *Cdkn2a/2b-Mtap* sub-region. These studies provided a detailed description of the genomic structure of the deletion region on RNO5, in particular of the *Ifna-Cdkn2a/2b-Mtap* sub-region.

Secondly, the functional role(s) of the DNA segment encompassing the *Ifna/Cdkn2a/2b-Mtap* region was analysed, by transfecting this DNA segment cloned in YACs into the tumor hybrid cells carrying deletion on RNO5. It was shown that the *Cdkn2a/2b* YAC clones but not the *Ifna* YAC clones elicit strong growth inhibitory effects on the hybrid cells. Three genes encoded by *Cdkn2a/2b* loci, *p15*, *p16* and *p19*, were analysed for their expressions in the immortalised YAC transfectant cells and in the anchorage independent cells. It was concluded that both *p15* and *p19* have important roles in immortalization while *p16* may regulate cellular anchorage dependence in this hybrid system.

Thirdly, genes expressed differently in the immortalized YAC transfectant and its derivative anchorage independent sub-clones were studied by cDNA RDA. In total, 79 transcripts were identified: the majority of them was found to be related to growth regulation, stress response/apoptotic functions, or to the function of the extracellular matrix (ECM). Our findings provided new insights into (1) the role of *Cdkn2a/p16INK4a* in the regulation of anchorage dependence, through the ECM protein fibronectin and its receptor integrin $\alpha5\beta1$, (2) the involvement of the ribosomal function and the rRNA processing in relation to *Cdkn2a/p19ARF*, and (3) the role of iron metabolism, particularly with respect to the ferritin heavy chain gene, *Fth*.

Keywords: cancer, anchorage independence, immortalization, tumor suppressor genes, *Cdkn2a*, *Cdkn2b*, RNO5, RDA, FISH, YAC transfection, YAC contig, PAC contig.

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