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EXPRESSION OF ONCOGENES DURING INDUCED DIFFERENTIATION OF HUMAN HEPATOCARCINOMA CELL LINE

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There was no detectable expression of c-*fos*, but a little c-*myc*, high c-*fms* and mederate high IGF-Ⅰ mRNA in the untreated human hepatocarcinoma cell SMMC-7721. After treatment with 10 μmol/L retinoic acid or 0.5 mmol/L dibutyryl cyclic-3′, 5′ adenosine monophosphate (db-cAMP), the c-*fos* was transiently expressed within 20—60 mins. If the treatment of RA or db-cAMP prolonged to 1—5 days, the transcriptions of c-*myc* were increased, reaching the highest level on the 2nd and 4th day. Simultaneously the transcriptions of c-*fms* and IGF-Ⅰ were gradually decreased. On the 5th day of the treatment, c-*fms* and IGF-Ⅰ mRNA were decreased to 32% and 14% respectively of the control (untreated cell) value by RA, and 35% and 22% respectively by db-cAMP. The biological significance of the above mentioned results was discussed.

Key words: Human hepatocarcinoma cell line, Induced differentiation, Oncogene.

It was discovered by our lab that all-trans retinoic acid (RA) and cyclic-3′, 5′ adenosine monophosphate (cAMP) induced the differentiation of SMMC-7721 human hepatocarcinoma cell line, reversed some of the malignant phenotypes of the cells,[1,2] and the expression of oncogene N-*ras* was decreased by RA.[3] In order to elucidate the mechanism of induced differentiation by RA and c-AMP, the effects of these two compounds on the expressions of other oncogenes, including c-*fos*, c-*myc*, c-*fms* and IGF-Ⅰ were further investigated. C-*fos* and c-*myc* code the transcription factors, also called DNA binding proteins, in cell nuclei. Both genes response very quickly to extracellular signals, and their expressions usually change within several or tens of miniutes. The change of c-*fos* expression is always

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very transient, but the expression of c-*myc* is comparatively perminent. C-*fms* codes the receptor of a growth factor, colony stimulating factor-1 (CSF-1). IGF-Ⅰ, so called insulin like growth factor Ⅰ, stimulates cell proliferation, and its gene may be seemed as an oncogene-like gene. Gu et al.[4,5] reported that genes of c-*myc*, c-*fms*, IGF-Ⅰ and IGF-Ⅰ receptor were over expressed in human primary liver cancer (PLC), so it is worth studying whether the changes of these gene expressions are opposite to those of PLC during the induced differentiation of hepatocarcinoma cells. In the present paper, the effects of RA and dibutyryl c-AMP (db-cAMP) on the expressions of c-*fos*, c-*myc*, c-*fms* and IGF-Ⅰ genes in SMMC-7211 human heptocarcinoma cell line were reptorted.

MATERIALS AND METHODS

Cell Culture of SMMC-7721

The cells were cultured in RPMI-1640 medium (GIBCO) according to the routine method established in our lab.[1] The final concentration of RA and db-cAMP (Sigma) were 10 μmol/L and 0.5 mmol/L respectively. Cells were cultured 20 mins to 24 hrs for the c-*fos* experiments and 1 to 5 days for the exprements of other genes.

Isolation of Cell RNA

The isothiocyanate (Fluka)-phenol-chloroform method[6] was used. The A260/A280 of the purified RNA was 20, and 2 clear homogenuous undegrated bands of 28S and 18S were shown after denatured electrophoresis on agarose gel.

RNA Electrophoresis and Transfer to Nitrocellulose Membranes

The classical method introduced in the book "Molecular cloning——A laboratry manual" was used.[7] The total RNA contents applied into each sample well of a electrophrotic plate were identical.

Preparation of Gene Probes

The α-^{32}P-dCTP (Amersham) labeled IGF-Ⅰ genes probe 1.0 kb, and c-*fos*, c-*fms*, or c-*myc* gene containing pBR322 plasmids were provided by Division of Biochemistry and Molecular Biology, Shanghai Cancer Institute. Using the serial methods described in reference.[8] The *E. Coli* transfected by these plasmids were amplified with LB plates followed by LB liquid medium, and the plasmids were isolated by SDS methods. After digested with restriction endonucleases (Pst 1 for the c-*fos* or c-*fms* containing plasmids, EcoR 1 and Cla 1 for the c-*myc* containing plasmid), the gene fragments were recovered on agarose gel electrophretic plates and the length of c-*fos*, c-*myc* and c-*fms* were 1.3, 1.8 and 1.6 kb respectively. The nick translation of the c-*fos*, c-*myc* and IGF-Ⅰ genes, and the random primed labeling of the c-*fms* gene were according to the manuals of Boehringer kit. The specific activities of the probes were not less than 1×10^8 cpm/μg.

Prehybridization, Hybridization on Nitrocellulose Membranes and Radioautography

The modified Imaizumi method[9] was used. After development of the X-film, the density of the hydridized spots were scanned by CS-910 scanner, and the gene expressions were dipicted as the area of the peaks.