Receptor-C_k regulates HMGCoA reductase gene in HL-60 cells

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Using human promyelocytic leukaemic HL-60 cell line as an archetype model, the present study was addressed to understand the role of Receptor-C_k in the modulation of sterol-response element (SRE) having DR-3 locus in the promoter region of HMGCoA reductase gene. Such a study unambiguously revealed that Receptor-C_k has the inherent capacity to regulate this SRE sequence in HL-60 cells. Based upon this study as well as our earlier findings, it is proposed that Receptor-C_k-dependent signalling may be of importance in leukaemogenesis.

UNAMBIGUOUS evidence now exists that cellular cholesterol homeostasis is maintained by the feedback mechanism involving the cholesterol molecule itself as an end-product repressor of genes having sterol response element (SRE) in their promoter region[1–5]. The importance of this cholesterol feedback control to human health was established by the findings which revealed a direct correlation between loss of this end-product control of cellular cholesterol metabolism and the genesis of various human diseases, especially atherosclerosis and cancer[6–8]. Cells from higher animals face the complex problem of not only sensing extracellular cholesterol but also intracellular oxysterol pool, that arises as a result of either uptake through passive diffusion or apoprotein B/E-specific LDL receptor or oxidation of cholesterol within cells[9,10]. Recent studies have identified these cholesterol sensors designated as Receptor-C_k for extracellular cholesterol[5] and LXR-α for intracellular oxysterols[11]. LXR-α has been shown to regulate the transcription of SREBP gene[12] and Receptor-C_k has been shown to regulate various genes involved in cholesterol homeostasis, cell growth and cell death through transcription factors having affinity for SRE sequence in their promoter regions[5]. Recently, a direct repeat (DR-3) SRE locus has been identified in the promoter region of two genes[13] coding for HMGCoA reductase and cholesterol ester transfer protein (CETP). This DR-3 SRE locus has been shown to be regulated by two transcription factors, SREBP and YY1 (ref. 13). These findings prompted us to explore the role of Receptor-C_k in the regulation of this unique DR-3 SRE locus in human promyelocytic leukaemic HL-60 cell line, recognized by us as an archetype cellular model[5,14] for the study because of following reasons: (i) Unable to express Receptor-C_k gene-product in its native state; (ii) exhibits deregulated mevalonate pathway; (iii) exposure of this cell to phorbol ester induces expression of Receptor-C_k gene product as well as differentiation into monocytes.

The human promyelocytic leukaemic HL-60 cell line (obtained from National Centre for Cell Science, Pune) was maintained in DMEM supplemented with antibiotics and 20% FCS. Cells were counted in a haemocytometer chamber and viability was determined by tripan-blue dye exclusion. Lipoprotein-deficient serum (LPDS) and 12-O-tetradecanoyl phorbol-13-acetate (TPA) were obtained from Sigma. TOPO Reporter and β-Gal Assay kits were obtained from Invitrogen.

HL-60 cells were seeded in a six-well plate at an initial density of about 5×10^5 cells/well in DMEM enriched with either 20% FCS or 20% FCS + TPA (100 μM) for 72 h at 37°C in 5% CO_2-atmosphere. At the end of this incubation period, the cells were harvested from each well and subjected to either protein extraction procedure or Leishman's stain for morphological examination under a light microscope. The protein extract of each well was subjected to SDS–PAGE followed by Western blotting. The expression of Receptor-C_k (69 kDa) was detected by employing polyclonal monospecific antibody against Receptor-C_k and immunodetection procedure reported earlier[15]. In another set of experiments, the HL-60 cells were exposed to DMEM + 20% FCS enriched with either TPA (32 nM) alone or TPA (32 nM) + antibody against Receptor-C_k for 72 h at 37°C in 5% CO_2-atmosphere. At the end of this incubation period, the cells were subjected to Leishman's stain and subsequently examined morphologically by a light microscope. The cells, in which the Receptor-C_k gene expression was induced, were labelled as Receptor-C_k (+ ve) cells and those in their native form which did not express Receptor-C_k gene were labelled as Receptor-C_k (– ve) cells. These two types of cells were used for transfection experiments. DR-3 SRE (ATGGTGCAGATGGTG; present in HMGCoA reductase gene) was incorporated into pGlow-TOPO vector with the help of TOPO cloning reaction according to the procedure given by Invitrogen. TOPO vector (without insert) was used as internal control. Confluent Receptor-C_k (+ ve) and (– ve) cells, seeded at a density of about 3×10^5 cells/well in six-well plates, were transfected by replacing growth medium (DMEM + ampicillin + 10% LPDS) with Escort-plasmid medium mixture (about 0.7 ml/3.5-cm well). The cells were incubated for 24 h at 37°C. At the end of this incubation period, the medium in each well was aspirated and replaced with growth medium (supplemented with 10% LPDS + ampicillin) containing either cholesterol (50 μg/ml) or TPA (32 nM) + cholesterol (50 μg/ml) or cholesterol (50 μg/ml) + antibody against Receptor-C_k protein (Ab*RC_k), and subsequently the cells were incubated for 24 h at 37°C in 5% CO_2-incubator. At the end of this incubation period the cells from each well were lysed and assayed for β-galacto-

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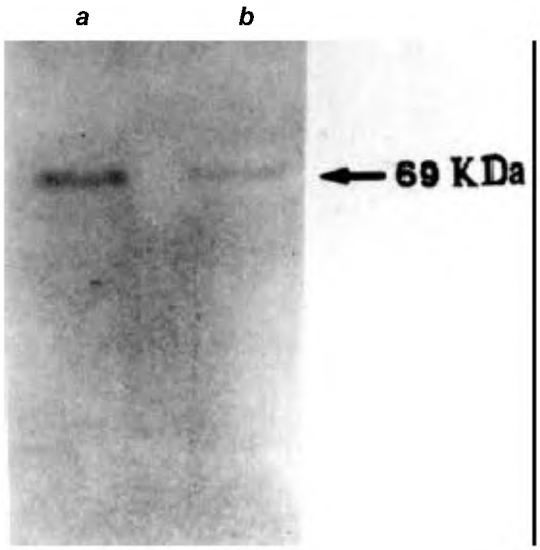

Figure 1. Expression of Receptor-C_k (69 kDa) in HL-60 cells exposed to either (*a*) TPA for 24 h or (*b*) TPA for 6 h.

sidase activity according to the standard method given by Invitrogen.

Exposure of HL-60 cells (which in their native state lack Receptor-C_k expression; Figure 1 *b*) to medium enriched with TPA resulted in the induction of Receptor-C_k gene expression (Figure 1 *a*). Further, exposure of these HL-60 cells to medium containing either TPA alone or TPA + Ab*RC_k revealed that HL-60 cells treated with (i) TPA alone were totally differentiated to monocyte phenotype (Figure 2 *a*); (ii) TPA coupled with Ab*RC_k exhibited promyelocytic population without any maturation or differentiation (Figure 2 *b*). These results unambiguously indicate that TPA-induced differentiation of HL-60 cells is mediated through Receptor-C_k-dependent signalling pathway. Exposure of Receptor-C_k (+ ve) cells to cholesterol exhibited 12-fold less SRE-dependent reporter-gene activity than Receptor-C_k (– ve) cells exposed to cholesterol (Table 1). This reduction could be restored in Receptor-C_k (+ ve) cells exposed to cholesterol coupled with Ab*RC_k (Table 1). However, Receptor-C_k (– ve) cells exposed to TPA coupled with cholesterol displayed three-fold reduction in SRE-dependent reporter-gene activity as compared to Receptor-C_k (– ve) cells exposed to cholesterol alone (Table 1). These results reveal that cholesterol-dependent activation of Receptor-C_k regulates the SRE-dependent reporter-gene activity.

There is now ample evidence to support the conclusion that loss of cholesterol feedback control of mevalonate pathway represents the most consistent molecular defect so far identified in the malignant and premalignant states[1]. A cell can respond to this defect in many ways: it can delay cell division until the damage is repaired; it can undergo apoptosis or it can progress without interruption through the cell cycle. Apoptosis has the inherent capacity to prevent malignant transformation because it removes

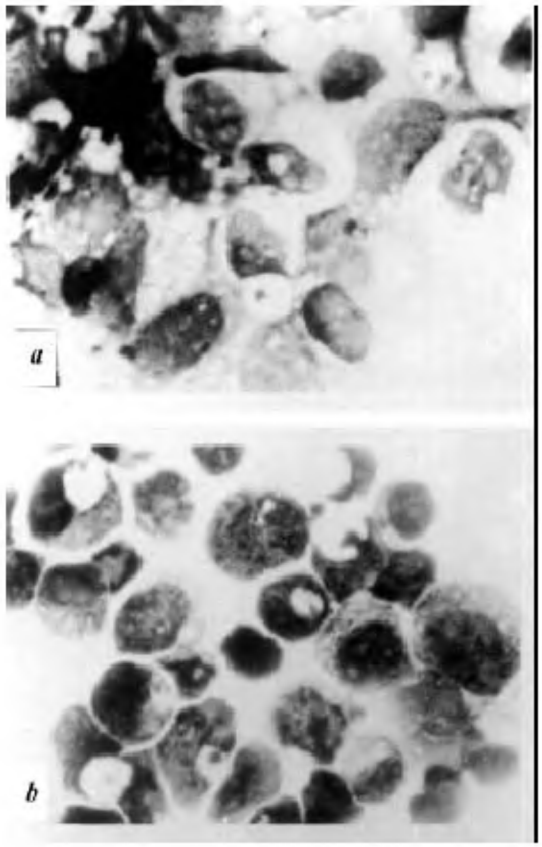

Figure 2. Representative photomicrograph showing (*a*) monocytic differentiation of HL-60 cells exposed to TPA, and (*b*) predominant promyelocytic population without differentiation of HL-60 cells exposed to TPA + antibody specific to Receptor-C_k (Ab*RC_k); (Leishman's stain X 1375).

Table 1. HMGCoA reductase promoter-dependent β-galactosidase reporter-gene activity

Transfected cell type	Exposure to DMEM + LPDS with additions	β-galactosidase specific activity (nm/mg/min)
Receptor-C_k (– ve)	–	6.5 ± 0.3*
Receptor-C_k (– ve)	Cholesterol	6.7 ± 0.2*
Receptor-C_k (– ve)	TPA + cholesterol	2.0 ± 0.05
Receptor-C_k (+ ve)	Cholesterol	0.5 ± 0.1
Receptor-C_k (+ ve)	Cholesterol + Ab*RC_k	6.8 ± 0.4

*Mean ± SD of experiments done in triplicate.

cells with genetic lesions. Abnormal apoptosis can initiate cancer development, both by accumulation of dividing cells and by obstructing the removal of genetic variants with enhanced potential. SRE/SRE-like sequences have been identified in the promoter regions of many genes[5] encoding several enzymes involved in the mevalonate pathway (such as HMGCoA synthase, HMGCoA reductase, farnesyl diphosphate synthase and squalene synthase); LDL-receptor (Apo B/E-specific); Bcl-2 (repressor of cellular apoptosis). The results reported here assume importance because of recent findings[5,15–18] that (i) Inability of

HL-60 cells to express Receptor-C_k is responsible for the elevated expression of genes coding for Bcl-2; cyclin D; c-myc; chimeric BCR-abl and Apo B/E-specific LDL-receptor in these cells; (ii) Receptor-C_k-dependent signalling is impaired in mononuclear cells from acute leukaemic patients; (iii) Defective Receptor-C_k-dependent signalling is observed in CNS tumours; (iv) HMGCoA-reductase inhibitor, Lovastatin, which is used extensively in the treatment of hypercholesterolemia, induced a potent apoptotic response in human neuroblastoma and acute myeloid leukaemia cells. Hence Receptor-C_k-dependent regulation of SRE present in HMGCoA reductase gene promoter may be of importance in the regulation of mevalonate pathway in human cells – a phenomenon of importance in leukaemogenesis/cancer.

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Vegetation mapping and slope characteristics in Shervaryan Hills, Eastern Ghats using remote sensing and GIS

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This communication discusses the various vegetation types distributed in the slopes of the Shervarayan hills and their mapping using remote sensing and GIS. Six major vegetation types, forest plantation and other land cover categories have been mapped. The slope map was prepared and overlaid on the vegetation-type map and the vegetation in different slope categories was identified. Sampling plots of 0.1 ha were established for each vegetation type in different slope categories. The woody species were measured at ≥ 30 cm gbh. The analysis shows that the forestland occupies a major part of the hill (49.50%) followed by villages (38.12%) and forest plantation (12.37%). The slope is directly proportional to the number of individuals, but indirectly proportional to the girth of the woody species. The data provided by the spatial distribution of the vegetation classes in different slope categories could help in the effective management/conservation of vegetation in the Shervarayan hills.

VEGETATION patterns are an integrated reflection of abiotic and biotic factors that shape the environment of a given land area[1,2]. Vegetation mapping plays a vital role in providing relevant information and therefore becomes a prerequisite for the effective management of natural resources, especially for the conservation of biodiversity[3] and is surrogate for ecosystems in conservation evaluations[4,5], and thus serves as a starting point of monitoring

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