Oxysterol receptor LXRα regulates SREBP gene expression in HL-60 cells exposed to differentiating agents

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 coding for HMGCoA reductase and Apo B/E-specific LDL receptor through a 125 kDa transcription factor (SREBP) having affinity for the conserved sterol regulatory element (SRE) present in the promoter region of these genes. The importance of this cholesterol feedback control to human health was established by the findings which indicated a direct correlation between loss of this end-product control of cellular cholesterol metabolism and the genesis of various human diseases, especially leukaemias. 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 apo B/E-specific LDL receptor or oxidation of cholesterol within cells. Recent studies have identified these cholesterol sensors designated as receptor-C4 for extracellular cholesterol and LXRα for intracellular oxysterols. LXRα has been shown to regulate the transcription of SREBP gene and receptor-C4 has been shown to regulate various genes involved in cholesterol homeostasis; cell growth (cyclin ‘D’, c-fos, c-myc, p-27, etc.), cell death (Bcl-2) through 125 kDa transcription factor (SREBP) having affinity for SRE sequence in its promoter region. Further, it was found that inhibitors of mevalonate pathway repressed LXRα activity and this repression was relieved by the addition of mevalonate or oxysterol or retinoic acid, but not by other products of the mevalonate pathway. In contrast, geranylgeraniol (GG-OH), another isoprenoid of mevalonate pathway, was itself an inhibitor of LXRα. An other dimension was added to the leukaemia research by the finding that blasts, derived from both ALL and AML patients, were unable to express SREBP gene product, although they had the capacity to express receptor-C4 gene product.

Based upon these above-mentioned findings and using human HL-60 promyelocytic leukaemic cell line (recognized as archetype cellular model to study myelopoiesis), the present work was addressed to study the effect of inhibitors of mevalonate pathway and agents shown to induce myelopoiesis upon the activity of LXRα as well as SREBP gene expression in HL-60 cells. The HL-60 human promyelocytic leukaemic cell line (obtained from National Centre for Cell Science, Pune) was maintained in RPMI-1640 medium supplemented with antibiotics and 20 per cent FCS. Cells were counted in a haemocytometer chamber and viability was determined by tripan blue dye exclusion. Escor transfection reagent, luciferase assay kit; vitamin ‘D3’, retinoic acid; mevastatin (specific inhibitor of HMGCoA reductase); lemonene (inhibitor of farnesyl transferase involved in Ras pathway) and FCS were purchased from Sigma. Luciferase reporter plasmid, TK-LXRE3-LUC (which contains the LXRE binding site) was a generous gift from David J. Mangelsdorf, Howard Hughes Medical Institute Dallas, Texas, USA. Specific antibody against SREBP gene product was purchased from Santa Cruz Biotech, USA.

HL60 cells were prepared for transfection, with luciferase reporter plasmid (containing the LXRE binding site) and β-glucosidase (β-gal) expression plasmid (as an internal control), by replacing the growth medium with Escor-plasmid medium mixture (about 0.7 ml/3.5 cm well). The cells were incubated for 5–6 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 20% of FCS + antibiotics) containing either no stimulus or mevastatin (30 μM) or lemonene (2.5 μM) or vitamin ‘D3’ (1 μM) or retinoic acid (1 μM), and subsequently the cells were incubated for 24 h at 37°C in 5% CO₂-incubator. At the end of this incubation period, the cells from each well were lysed and analysed for luciferase and β-gal activity. Data are presented as luciferase values normalized for β-gal activity and each value represents the mean of three separate observations. In another set of experiments, HL-60 cells were exposed either to no stimulus or mevastatin (30 μM) or lemonene (2.5 μM) or vitamin ‘D3’ (1 μM) or retinoic acid (1 μM) and incubated for 24 h at 37°C in 5% CO₂-incubator. At the end of this incubation period, the protein extract of cells from each well was subjected to SDS-PAGE followed by Western blotting. The immunodetection of the Western blots was carried out using specific antibody against SREBP gene product and employing the procedure reported earlier.

Compared to the basal activity of LXRα in HL-60 cells, exposure of these cells to mevastatin (blocker of HMGCoA reductase – a rate-limiting enzyme in mevalonate pathway) resulted in significant reduction in LXRα activity (Figure 1a). Exposure of these cells to lemonene (blocker of farnesyl transferase – having a crucial role in Ras pathway), vitamin ‘D3’ and retinoic acid resulted in increased activity of LXRα compared to the basal values (Figure 1a). Compared to the basal expression SREBP gene product in HL-60 cells, exposure of these cells to

Figure 1. Regulation of oxysterol receptor LXRα activity (a) and SREBP gene expression (b) in HL-60 cells exposed to indicated agents.
lemonene, vitamin ‘D3’ and retinoic acid resulted in increased expression, whereas exposure of these cells to mevastatin resulted in decreased expression of this gene product in HL-60 cells (Figure 1 b). These results ambiguously reveal that LXRα regulates SREBP gene expression in HL-60 cells and this LXRα activity is also influenced by agents known to induce myelopoesis in this cell line.

The size of any hematopoietic cell compartment is maintained by a finely orchestrated balance between input (proliferation) and output (differentiation + apoptosis) processes. Abnormalities in any of these processes give rise to blood diseases, especially leukemia. Since extracellular cholesterol sensor ‘receptor-C4’ has been shown to regulate genes involved in the cell cycle, cell death as well as cholesterol homeostasis, through SREBP gene product having affinity for sterol regulatory element (SRE) present in the promoter region of these genes, studies were addressed to understand its role in leukemia. On the basis of these studies, it was recently proposed that receptor-C2-dependent signalling is impaired in chronic state of leukemia by the inability of these cells to express receptor-C2 gene, whereas receptor-C2-dependent signalling is impaired in the acute state of leukemia by the inability of these cells to express SREBP gene product. Further, oxysterol receptor LXRα has been shown to upregulate the transcript of 125 kDa SREBP gene. It is interesting to note that (a) Constitutive activity of LXRC-XXR requires mevalonate biosynthesis, whereas oxysterols directly activate LXRC within cells; (b) Receptor-C2 gene expression could be induced in HL-60 cells (that are unable to express Receptor-C2 gene in their naïve state) by either phorbol esters or vitamin D3 + retinoic acid, whereas vitamin ‘D3’ or retinoic acid alone were ineffective; (c) Using HL-60 cell line as an in vitro model, it was shown that retinoic acid and vitamin ‘D3’ interact additively with respect to the inhibition of cell growth, DNA synthesis, induction of cell differentiation and loss of cell clonogenicity. The results reported here reveal that both retinoic acid and vitamin ‘D3’ are able to upregulate LXRα activity as well as SREBP gene expression in HL-60 cells (Figure 1).

This finding raises the question regarding the inducibility of SREBP gene expression in blasts derived from both ALL and AML patients by either vitamin ‘D3’ or retinoic acid or to what extent inducibility of SREBP gene expression, in cells derived from AML and ALL patients, can contribute to the treatment of acute leukemia patients? In this context, it is interesting to note that increased sensitivity in AML to lovastatin-induced apoptosis has been observed. Further, leukemia cells from AML patients exhibit decreased feedback regulation of LDL-receptor activity by sterols. However, the results reported here provide evidence that differentiating agents (such as vitamin ‘D3’ and retinoic acid) have inherent capacity to regulate intracellular oxysterol receptor LXRC, which in turn, regulates SREBP gene expression in HL-60 cell. It is also pertinent to note that (a) activation of LXRα results in the down-regulation of genes coding for c-myc and Bcl-2 (ref. 19); (b) differentiating agents (vitamin ‘D3’; retinoic acid; phorbol esters, etc.) induce differentiation of HL-60 cells through their ability to down-regulate c-myc gene expression in these cells; (c) a number of observations suggest that the c-myc expression is critical to maintaining HL-60 cells in an undifferentiated state and that inhibition of this c-myc expression alone results in the terminal differentiation of HL-60 cells. Further, the HL-60 cells do not possess the receptors specific for retinoid acid. Hence retinoic acid-dependent differentiation of HL-60 cells may be mediated through its ability to activate LXRC, resulting in the down-regulation of c-myc gene expression in these cells. Keeping in view these observations, we propose that LXRC may have an important role in leukemic haemopoiesis.


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