

tained from fibrosarcoma cells, meaning that despite the heterogenous nature of sarcoma tissue, the spectral pattern of the predominant cell type is maintained. Phospholipid metabolites, fucose block volume and triglyceride to a lesser extent, appear to reflect characteristics of the tissue, its grade and its type, thus functioning as 'NMR markers' in the study of sarcoma tissue.

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Receptor-C_k controls cholesterologenesis and DNA replication in HEP G2 cells

D. Kaul* and M. Kaur

Department of Experimental Medicine and Biotechnology, Post Graduate Institute of Medical Education and Research, Chandigarh 161 012, India

The present study, addressed to understand the molecular link between LDL, cholesterologenesis and DNA replication in Hep G2 cell line, revealed that the inhibitory effect of LDL upon cellular DNA synthesis, cholesterol synthesis and apo B-specific LDL-receptor gene expression at the transcriptional level, was mediated entirely through the activation of Receptor-C_k and not through its conventional apo B-specific LDL-receptor pathway. Based upon these experiments, we propose that Receptor-C_k controls the cellular cholesterologenesis as well as DNA replication and hence, it may have a crucial role in hepatic pathophysiological process.

RECENT studies addressed to understand the paradoxical interaction of lipoproteins with human platelets revealed the existence of a unique and a novel cell surface 69 kDa glycoprotein designated as Receptor-C_k based upon its characteristic of having high affinity for cholesterol moiety in various types of lipoproteins as well as intrinsic tyrosine kinase activity responsible for intracellular signaling¹⁻³. This Receptor-C_k was not only shown to be ubiquitously present in various human cells/organs but also was conspicuously and selectively absent in

various types of leukemic cell lines as well as patients³. Further, Receptor-C_k-dependent signaling was also shown to regulate mevalonate pathway and apo B-specific LDL-receptor gene at the transcriptional level in human lymphocytes⁴. Since Hep G2 cell line also has the ability to express this Receptor-C_k (ref. 3) and apo B-specific LDL-receptor responsive to LDL, the present study was undertaken to understand the role of Receptor-C_k in the regulation of endogenous cholesterol synthesis, DNA synthesis and transcriptional expression of apo B-specific LDL-receptor gene in this cellular model.

Hep G2 cell line was obtained from the National Facility for Animal Tissue and Cell Culture, Pune, India. ³H-thymidine and ³H-water were purchased from Bhabha Atomic Research Center, Bombay. Nucleic acid kit was obtained from Boehringer Mannheim (Germany). Polyclonal monospecific antibody against Receptor-C_k (Ab-RC_k) was raised in our laboratory. Oligonucleotide probe for apo B-specific LDL-receptor gene was obtained from Molecular Medicine Unit, King's College London, UK.

Low density lipoprotein (LDL) fraction was obtained from the plasma of normal healthy human subjects by using the standard gradient ultracentrifugation method⁵.

Receptor-C_k (69 kDa glycoprotein) was purified from human platelets to high purity by the method reported earlier³ and rabbits were immunized with this preparation emulsified 1:1 with Freund's complete adjuvant given intraperitoneally. Two weeks later, the same amount of protein (25 µg) emulsified with Freund's incomplete adjuvant, was injected intraperitoneally. These rabbits then received weekly i.p. injection of the same preparation by booster dose 4 days before blood was drawn from the animals. The antibodies from serum were purified by precipitation with ammonium sulphate

*For correspondence.

and DEAE-matrix chromatography. Protein was measured in the purified antibody (designated Ab-RC_k) solutions by spectrophotometry⁶.

Hep G2 cell line was maintained in DMEM enriched with 10% fetal calf serum and antibiotics in 5% CO₂ atmosphere at 37°C. For the present study, the cells from the confluent culture were seeded at a density of approximately 10⁵ cells/well in multiwell culture plates. These cells were exposed to DMEM containing 10% LPDS for 24 h prior to addition of various stimulants.

At the end of 25 h synchronization period, the Hep G2 cells were exposed to DMEM enriched with the following additions: a) LPDS (10%) alone; b) LPDS (10%) + LDL (25 µg/ml); c) LPDS (10%) + LDL (25 µg/ml) + Ab-RC_k; d) LPDS (10%) + Ab-RC_k alone for 2 h at 37°C. At the end of this incubation period, the cells were again incubated under the above-mentioned experimental conditions along with ³H-thymidine (2.5 µCi/well) for another 6 h. At the end of this incubation period, the cells in each well were washed and ³H-thymidine incorporation into DNA was determined subsequently by the use of standard method⁷. An aliquot of cell suspension was also used for counting the number of cells/well.

At the end of 25 h synchronization period, the Hep G2 cells were exposed to DMEM enriched with the following additions: a) LPDS (10%) alone; b) LPDS (10%) + LDL (25 µg/ml); c) LPDS (10%) + LDL (25 µg/ml) + Ab-RC_k; d) LPDS (10%) + Ab-RC_k alone for 2 h at 37°C. At the end of this incubation period, the cells were again exposed to the above-mentioned conditions along with ³H-water (50 µCi/ml) and incubated for another 6 h. Incubation was terminated by washing the cells with ice cold buffered saline. Cells were scraped off from the culture wells and harvested by centrifugation and lipids were extracted with chloroform: methanol (2:1). The samples were processed for the estimation of ³H-water incorporation into cholesterol using standard method⁸.

Total RNA from Hep G2 cells exposed to: a) LPDS (10%) alone; b) LPDS (10%) + LDL (25 µg/ml); c) LPDS (10%) + LDL (25 µg/ml) + Ab-RC_k; d) LPDS (10%) + Ab-RC_k alone was isolated by acid-guanidinium thiocyanate phenol chloroform extraction method⁹. The total RNA isolated was blotted on to the nylon membrane by the use of Manifold I and II (Schleicher and Schuell) 96 well dot blot apparatus, which was subsequently subjected to hybridization with the DIG-labelled apoprotein-B specific LDL-receptor cDNA probe at 68°C (ref. 10). After hybridization, the membrane was subjected to stringent washings. These were washed twice for 5 min each at room temperature with at least 50 ml of 2 × SSC buffer containing 0.1% (w/v) SDS and with 0.1 × SSC containing 0.1% (w/v) SDS at 68°C for 2 × 15 min. After washing, the mem-

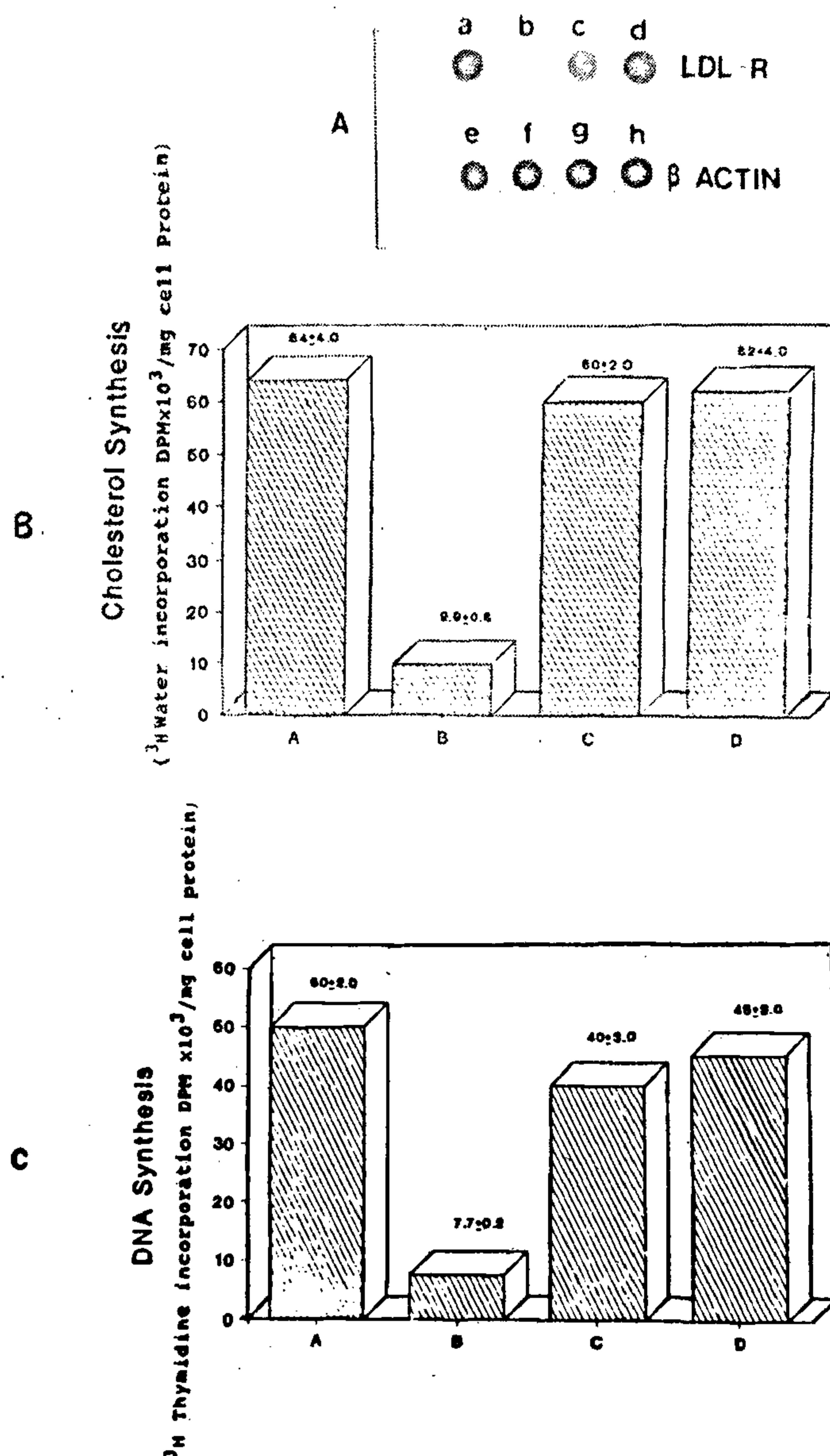


Figure 1. A, Transcriptional expression of apo B-specific LDL-receptor gene in cells exposed to a) LPDS alone; b) LPDS + LDL; c) LPDS + LDL + Ab-RC_k; d) LPDS + Ab-RC_k. (e-h) represents expression of β -actin gene as an invariant control. B, Cholesterol synthesis in cells exposed to medium containing a) LPDS alone; b) LPDS + LDL; c) LPDS + LDL + Ab-RC_k; d) LPDS + Ab-RC_k. C, DNA synthesis in cells exposed to medium containing a) LPDS alone; b) LPDS + LDL; c) LPDS + LDL + Ab-RC_k; d) LPDS + Ab-RC_k.

brane was processed for the detection of hybridized DNA by DIG detection kit (Boehringer Mannheim). Expression of β -actin gene, a housekeeping gene, was examined as an invariant control.

Exposure of Hep G2 cells to medium enriched with LDL resulted in downregulation of cholesterol synthesis, DNA synthesis as well as apo B-specific LDL gene transcription as compared to the cells exposed to medium enriched with LPDS (Figure 1A-C). This phenomenon was totally abolished when the cells were exposed to specific antibody against Receptor-C_k alone or together

with LDL (Figure 1A_{cd}, 1B_{cd}, 1C_{cd}). These results unambiguously revealed that LDL-dependent activation of Receptor-C_k is responsible for the down regulation of cholesterol synthesis, DNA synthesis and apo B-specific LDL-receptor gene transcription (Figure 1A_b, 1B_b, 1C_b).

Cholesterol-feedback control responsible for cholesterol homeostasis within the cell is achieved by cholesterol-dependent feedback regulation of at least two sequential enzymes in the mevalonate pathway, HMG CoA synthase and HMG CoA reductase as well as apo B-specific LDL-receptor¹¹. This cholesterol-dependent feedback regulation at the transcriptional level takes place through a conserved sterol regulatory element (SRE) present in the promoter region of the genes coding for the enzymes HMG CoA reductase and HMG CoA synthase as well as apo B-specific LDL-receptor gene¹². Using human promyelocytic leukemic cell line (HL-60) as an archetype cellular model (which is unable to express Receptor-C_k, whereas this cell line exhibits overexpression of conventional apo B-specific LDL-receptor), we have unambiguously shown that Receptor-C_k-dependent signalling regulates genes coding for HMG-CoA synthase, HMG-CoA reductase, apo B-specific LDL-receptor, Cyclin D (initiator of cell replication), Bcl-2 (suppressor of apoptosis) and mitotic inhibitor P²⁷ (refs 13, 14). Further, using human platelets as a cellular model (being a nucleated cell and unable to express apo B-specific LDL-receptor but able to express Receptor-C_k), we have shown that Receptor-C_k regulates cholesterol synthesis in this cell type also¹⁵. Consequently the results reported here are in conformity with our earlier results in other cellular models such as human lymphocytes, HL-60 cells and human platelets^{4,15,16}. We have recently shown that Receptor-C_k-dependent signalling regulates a 47 kDa factor having specific affinity for SRE sequence^{17,18} leading to the regulation of apo B-specific LDL-receptor gene. Further, a lot of evidence has accumulated to suggest a direct correlation between cholesterol synthesis, DNA replication and cell growth¹⁹. The results reported here also unambiguously show that a direct correlation exists between cholesterol synthesis and DNA synthesis (Figure 1). It is interesting to note that inhibitory effect of LDL upon cellular, cholesterol synthesis, DNA synthesis as well as transcriptional expression of apo B-specific LDL-receptor gene, is abolished when cells are pre-exposed to antibody specific to Receptor-C_k, thereby indicating that this LDL-dependent inhibitory effect is mediated through Receptor-C_k and not through its conventional apo B-specific LDL-receptor. This view is strengthened by various observations reported earlier which argue strongly against the apo B-specific LDL-receptor-dependent regulation of cellular cholesterol synthesis pathway²⁰. In conclusion, the data reported here provides strong evidence to support the view that LDL-dependent Receptor-C_k activation is

responsible for the regulation of cholesterol synthesis, DNA replication as well as cell growth.

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Chhota Shigri Glacier: Its kinematic effects over the valley environment, in the northwest Himalaya

Surendar Kumar

Wadia Institute of Himalayan Geology, Dehra Dun 248 001, India

The relation of surface lowering, net balance and flow dynamics reflects that vertical component of ice flow is downward in and around the equilibrium line while it is upward in the lower part (ablation zone) of Chhota Shigri Glacier. The submergence velocity in accumulation area is higher than the rise of surface and emergence velocity is lower than that of negative net balance. The basal sliding velocity is responsible for the movement of the glacier. The over-extension of the glacier at average snout position is one of the factors in controlling the temperature variation of the main Chandra river valley.

THE Chhota Shigri Glacier valley is a 9 km long narrow valley with about 8.75 km² of accumulation area which