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Role of caveolin in the regulation of cell cycle by insulin

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Progression through the cell cycle is regulated by the ordered activation of cyclin dependent kinases (cdks). In yeast, a single cdk, cdc2/CDC28, associates with multiple cyclin partners to regulate both the START and G2/M checkpoints, whereas in higher eukaryotes, a large family of cyclin dependent kinases (cdks) have so far been identified, each of which appears to regulate discrete cell cycle functions (3,4). Cdks coordinated cell cycle checkpoints, a series of biochemical pathways that ensure that the initiation of cell cycle events occur only after successful completion of others (5,6). Passage from G1 into S phase requires the activation of cyclin D1-associated cdk4 and cdk6 (7-10) in addition to cyclin E/cdk2 (11), both of which contribute to phosphorylation of the retinoblastoma protein, pRB (11-13).

Serum and growth factor (insulin, PDGF, EGF etc.) signaling to discrete transcription factor targets is coordinated by evolutionally conserved modular intracellular signaling kinase cascade. Transcription factor such as c-fos and c-jun may induce the cyclin D1 gene and thereby enhance S phase entry. Thus, c-fos was shown to induce the cyclin D1 gene. The cyclin D1 gene is inhibited during overexpression of caveolin-1 as a result of repression of the cyclin D1 promoter. The DNA sequences required contain the T-cell factor

(TCF)/lymphoid enhancer factor-1 (LEF-1)-binding site. The repression of the cyclin D1 gene by caveolin-1 contributes to the inhibition of cellular transformation (14). The expression of caveolin-1 is significantly reduced in human breast cancer cells compared with their normal mammalian epithelial counterparts. Caveolin-1 effects on the regulatory mechanism of cell growth and reduces not only cell growth but also tumorigenecity in vitro is consistent with the oncogenic transformation of the cells being responsible for the lack of caveolin-1 expression and this transcriptional inhibition relating functionally to the tumor phenotype. Cyclin A/ cdk2 activation is essential for S phase progression (9,11,15) and both cyclin A/cdc2 and cyclin B/ cdc2 are required for the transitions through G2/M (15). The progression of quiescent cells from the G0 through the G1 phase of the cell cycle is orchestrated by interaction between components of the cell cycle regulatory apparatus (13,16). The induction of the G1 phase regulatory cyclins, the cyclin-dependent kinase phosphatases, and the E2F-responsive genes contributes to the continued passage of the cell through G1 and into the S phase (17). Cells respond to extracellular signals by transmitting intracellular instructions to coordinate appropriate responses.

Unlike other growth factor receptor tyrosine kinases (GF-RTKs), the insulin receptor (IR) utilizes a family of soluble adaptor substrates (IRSs), to initiate its signaling program. Most of GF-RTKs regulate long-term events such as cell differentiation and mitogenesis. A major function of the IR is the acute regulation of glucose metabolism in muscle and fat cells (18). Insulin is an essential peptide hormone that regulates metabolism, growth, and differentiation. Biological actions of insulin are initiated when insulin binds to its cell surface receptor (18). The propagation of information from the insulin receptor culminates in diverse effects such as increased glucose transport, mitogenesis, and regulation of enzymatic pathway. Generally, insulin signaling is divided in

two steps. Short-term effects of insulin such as regulation of metabolism are coordinated by PI 3-kinase pathway. While long-term effects of insulin is related to MAPK pathway. Among the pathways often used to transduce these signals are through the highly conserved mitogen-activated proteins (MAPK) or extracellular signal regulated protein (ERK) cascades. These cascades are found in all eukaryotic organisms and consist of a three-kinase module that includes a MAPK, which in turn is activated by a MEK kinase (MEKK). This pathway stimulates to mitogenic signals and can be blocked by inhibiting it (19,20). The p42/p44 MAPK (ERK-1 and -2) cascade plays a pivotal role in the re-entry of fibroblasts into the cell cycle and in a variety of other processes, including differentiation of PC12 cells into neutrites in response to nerve growth factor (NGF) (21,22). ERK1/2 are re-localized from the cytoplasm to the nucleus upon stimulation (23-25). In fibroblast, a correlation exists between the mitogenic potency of a stimulus and its ability to trigger MAPK translocation. Comparison of the kinetics of MAPK activation and nuclear translocation suggests that it is the active form of MAPK that translocates into the nucleus (26,27). The ERK/MAPK cascade is also regulated by the relative abundance of the caveolin proteins (28-30).

Caveolae are 50-100nm vesicular invaginations of the plasma membrane. It has been proposed that caveolae participate in vesicular trafficking events and signal transduction processes (31-33). Caveolin, a 21-24 kDa intergral membrane protein, is a principal component of caveolae membranes in vivo (31, 34). Caveolin is expressed by at least three genes whose products are referred to as caveolin-1, caveolin-2, and caveolin-3. Caveolin-1 exists in two different isoforms, called alpha-caveolin-1 and beta-caveolin-1, which are produced by alternate translation of the same mRNA.

The tissue distribution of the caveolin-1 and caveolin-2 isoforms differs from

caveolin-3, because caveolin-1 and caveolin-2 are found predominantly in adipocytes, smooth muscle cells, and epithelia, whereas caveolin-3 is found predominantly in muscle cell types (cardiac and skeletal) (35, 36). It has been proposed that caveolin family members function as scaffolding proteins (37,38) to organize and concentrate specific lipids (cholesterol, GPI, glycolipid, and sphingolipid) and lipid-modified signaling molecules (Src-like kinases, H-Ras, eNOS, MAPK, and G-proteins) (38,39) within caveloae. In support of this idea, caveolin-1 binding can functionally suppress the GTPase activity of hetero-trimeric G-proteins and inhibit the kinase activity of Src-family tyrosine kinases through a common caveolin domain, termed the caveolin-scaffolding domain. Various functions of caveolae have now been proposed. Most common hypotheses are that caveolae function in protein sorting, transcytosis, cell signaling, and potocytosis (31).

Despite these reports, the molecular mechanisms by which caveolin regulates the cell cycle are mainly known in cav-1 and cav-3, and almost nothing is known about any regulatory role for cav-2, except structural role for formation of caveolae. In this study, we studied whether caveolin-2 can regulate the cell cycle and how caveolin-1 and -2 differently regulate the cell cycle through differential mechanism. To investigate a role/function (regulatory, not like known structural role) of caveolin-2 compared to the well defined inhibitory regulation of caveolin-1 in various cellular signal transduction including growth factors, Hirc-B cells (Human insulin receptor overexpressed rat 2 fibroblast cells) having endogeneous cavolin-2 as a major caveolin were tested for the regulatory fuction of caveolin-2 in the interaction with other signaling molecules in cell cycle regulation, and compared to the cavolin-1 transfected Hirc-B cell. Our results showed that the direct interaction of ERK and caveolin-2 and that the cav-2 regulate a positive way in the cell cycle of Hirc-B cells when compared to

caveolin-1.

Previously, Weinberg (16) reported that the cyclin D1 gene encodes the regulatory subunit of the holoenzyme that phosphorylates and inactivates the pRB protein, thereby promoting entry into the DNA synthetic phase of the cell cycle. Caveolin-1 expression is reduced in a variety of tumor types, whereas increasing the level of caveolin-1 levels can suppress the transformed phenotype. This is consistent with its antagonism of Ras-mediated cell transformation, in which caveolin-1 expression dramatically inhibited both Ras/MAPK-mediated and basal transcriptional activation of mitogen-sensitive promoter (28,29). Generally, downregulation of caveolin-1 expression is sufficient to drive oncogenic transformation and constitutively activate the p42/p44 MAPK cascade. It has been also reported that caveolin-1 mutants repressed the cyclin D1 promotor activity (13). Despite these results, mainly focused/concentrated/ reported on cav-1, the function of caveolin-2 is not well known except a structural role for caveolae formation compared with caveolin-1.

Insulin treatment in Hirc-B cells showed 6 fold increase in S phase. The increased S phase upon insulin treatment in Hirc-B cells was inhibited by caveolin-1 transfection. We further observed that caveolin-1 transfection in Hirc-B cell inhibited on G1/S phase transition and thus, induced G1 phase arrest. This observation is in a good accordance with many previous reports showing that the role of caveolin-1 as a negative regulator in the cell cycle.

Interestingly when we examined caveolin-2 expression level upon insulin treatment, the expression was increased with a time and insulin dose-dependent manner and caused the colocalization of caveolin-2 and ERK in the perinucleus in Hirc-B cells. Furthermore, cav-2 directly interacted with ERK and colocalized with ERK into the perinucleus of Hirc-B cells upon insulin treatment.

Thus, our data suggest that caveolin-2 acts as a positive regulator directly

interacting with ERK as in the cells undergoing carcinogenesis. The cell cycle regulating factors (cdc and cdk families) on G1 S transition might be modulated by caveolin-1 and -2 as negative and positive regulators, respectively in Hirc-B cells. Accordingly, our finding of the differential role of caveolin-2 compared to cav-1, unlike updated/recent reports about a mere structural role of caveolin-2, opens up many exciting investigation regarding caveolin-2 functions in cell cycle as well as in interaction of other signaling molecules as yet to be revealed.

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