Immunocytochemical detection of p21ras, Raf-1, ERK1/MAP kinase and PKC isoforms in a 20-methylcholanthrene-induced transformed murine embryonal fibroblast cells in culture

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An immunocytochemical study using antibodies against p21ras, Raf-1, MAP kinase/ERK1 and PKCα, β, γ, δ, ε, ζ, isoforms were performed on a 20-methylcholanthrene-induced transformed murine embryonal fibroblast cells in both *in vitro* and *in vivo* growth conditions. Altered expression of p21ras, Raf-1, MAP kinase in this particular cell line strongly supported the previous findings of the activation of one component of signal transduction under the influence of the other in the MAP kinase cascade of signal transduction during neoplastic transformation and which also seemed to be involved in CNCI-PM-20 cell line. The altered expression of PKCα, β, and δ was thought to be an epigenetic event occurring under the indirect influence of other changes in these cells. Host physiology and metabolism did not have much impact on the expression of these gene products after biological incubation of these cells in syngenic host.

Key words: Chemical carcinogenesis, components of MAP kinase cascade, PKC isoforms.

Overexpression of several genes implicated in the mitogen-activated protein kinase signaling cascade (mitogen-activated protein kinase, MEK-MAP kinase, Raf-1, Ras) seemed to be most likely responsible for initiated cells acquiring a proliferating phenotype, which facilitated the accumulation of structural changes in additional genes resulting in the generation of autonomously growing preneoplastic and neoplastic lesions [12]. The highly conserved family of ras genes in the MAP kinase cascade had been detected as transforming genes in a wide variety of naturally occurring tumors [16] and in *in vivo* and *in vitro* experimental models after carcinogenic insult [4, 32]. Although multiple ras effector pathways had been identified, the Raf protein kinases which lied downstream of ras were believed to be the primary mitogenic effectors [23] and also the raf protein were upregulators of the mitogen activated protein kinases (MAPK/Erk) [8, 11]. The constitutive upregulation of this pathway by oncogenic ras was thought to promote cellular transformation [4].

Protein kinase C, a ubiquitous family of eleven related isoforms were another group of signal transducing molecules deeply implicated in carcinogenesis [3]. Protein kinase C isoforms were usually divided into three main classes; classical PKC (α, β, γ), new PKC (δ, ε, η, θ) and novel PKC (ζ, λ/t). The level of total PKC and phosphorylated PKC got altered in several types of malignancies [3] and one important concomitant of PKC activation was the intracellular redistribution, overexpression and downregulation of different PKC isoforms in different compartment of the transformed cells in comparison to non transformed cells [21].

The present study was undertaken to identify the status of the various components of MAP kinase cascade (MAP kinase, Raf-1, Ras) as well as non phophorylated forms of different protein kinase C isoforms α, β, γ, δ, ε, ζ to determine how and at what extent the expression of these components behaved during the stepwise development of a 20-

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