687 bases. Amino acid sequence of the tomD gene product exhibited 71.1% identity with that of 4-oxalocrotonate decarboxylase from C. testosteroni TA441.

PI3K-PKCε signaling pathway is essential for the p21WAF1/Cip1 expression by apicidin

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We previously reported that PKC signaling event was closely involved in the expression of $p21^{WAF1/Cip1}$ by apicidin, a novel histone deacetylase inhibitor. In this study, we extended our study to elucidate the upstream signaling pathway for the expression of $p21^{WAF1/Cip1}$ by apicidin. The expression of $p21^{WAF1/Cip1}$ by apicidin appears to be mediated by PKCε, because the expression of dominant negative PKCε significantly attenuated the activation of $p21^{WAF1/Cip1}$ promoter via Sp1 sites and also inhibited the protein level of $p21^{WAF1/Cip1}$. And antisense oligonucleotide against PKCε decreased PKCε expression and inhibited the expression of $p21^{WAF1/Cip1}$, indicating that PKCε signaling event is essential for the expression of $p21^{WAF1/Cip1}$ by apicidin. Next, we examined the involvement of PI3K signaling pathway, a possible candidate upstream molecule of PKCε. LY294002 and wortmannin, a well known PI3K inhibitors, attenuated the activation of $p21^{WAF1/Cip1}$ promoter via Sp1 sites and also inhibited the protein level of $p21^{WAF1/Cip1}$. The expression of dominant negative PI3K abrogated the activation of $p21^{WAF1/Cip1}$ promoter, suggesting that the PI3K signaling event was deeply involved in the apicidin-induced $p21^{WAF1/Cip1}$ expression. And apicidin-mediated PKCε signaling event might be regulated by PI3K signaling pathway, since the expression of $p21^{WAF1/Cip1}$ by PDBu, a PKC activator, was not inhibited by the PI3K inhibitors and membrane translocation of PKCε in response to apicidin was blocked by the PI3K inhibitor. However, the $p21^{WAF1/Cip1}$ expression by apicidin appears to be independent of the histone hyperacetylation, since apicidin-induced histone hyperacetylation was not affected by PI3K inhibitors, suggesting that the expression of $p21^{WAF1/Cip1}$ by apicidin might have been mediated by a mechanism other than chromatin remodeling through the histone hyperacetylation. Taken together, these results suggest that the PI3K-PKCε signaling pathway plays a pivotal role in the expression of the $p21^{WAF1/Cip1}$ by apicidin.

MALDI-TOF MS Approach to Identify the E6AP-interacting factors in HeLa cervical cancer cells

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Cervical cancer is one of the leading causes of female death. Human papillomaviruses have been recognized as the primary cause of cervical cancer. Viral oncoproteins are selectively retained and expressed in carcinoma cells infected with human papillomavirus and cooperated in immortalization and transformation of primary keratinocytes. E6 associated protein (E6AP) is a 100 kDa cellular protein which mediates the stable association of the high-risk HPV E6 oncoprotein with tumor suppressor protein p53, resulting in the degradation of p53. E6AP was known as E3 ubiquitin-protein ligase, which has been proposed to play a role in defining the substrate specificity of the ubiquitin-proteasome degradation. In order to identify the E6AP-interacting molecules, HeLa cervical carcinoma cells having HPV type 18 genome, was used. We have produced his tagged E6AP and E6AP-Ni2+-NTA-affinity column was prepared to obtain E6AP-interacting proteins. The E6AP-interacting proteins were resolved in 2D-gel and analysed by matrix-assisted laser desorption/ionization (MALDI/TOF). Among 17 proteins identified in 2D patterns of