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PDF文档

利用FRET技术在活细胞内观察EGF对PKA作用的时空成像

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cAMP依赖的蛋白激酶（ protein kinase A, PKA）在细胞生长与分化过程中扮演重要角色，特别是在调节Ras信号通路引起的细胞增殖效应中起着重要作用。为了在活细胞内动态观察表皮生长因子（epidermal growth factor, EGF）对PKA的作用，采用一种可以检测PKA酶活性的报告蛋白（A-kinase activity reporter, AKAR）——这种报告蛋白是利用荧光共振能量转移（fluorescence resonance energy transfer，FRET）原理设计的，使其在人类肺癌细胞（ASTC-a-1）中稳定表达。加入EGF刺激因子后，随时间变化的成像分析显示出在活细胞生理条件下被EGF作用的PKA酶活性变化的时空信息。这些资料为EGF作用PKA提供了直接的实时证据。

SPATIO-TEMPORAL IMAGING OF EGF-INDUCED ACTIVATION OF PROTEIN KINASE A BY FRET IN LIVING CELLS

The cAMP-dependent protein kinase-A (PKA) is an intracellular enzyme with serine- threonine kinase activity that plays a key role in cell growth and differentiation. Epidermal growth factor (EGF), a well-characterized polypeptide cell growth factor, has been demonstrated to induce PKA activation through the ligation of EGFR. To investigate the spatial and temporal dynamics of PKA activation by EGF, we visualized the PKA activation in living cells using a fluorescent indicator (AKAR) composed of two green fluorescent protein (GFP) variants, cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP), joined by a phosphoamino acid binding domain (14-3-3) and a consensus substrate for protein kinase-A (PKA). Phosphorylation of the consensus substrate by PKA activation resulted in the transfer of energy from excited CFP to YFP within the AKAR molecule. A human lung cancer cell line (ASTC-a-1) that stably expressed the AKAR indicator protein was established and characterized. After treatment with EGF, the activation dynamics of PKA was visualized in living cells by fluorescence microscopic imaging system. Time-lapse imaging analysis reveals the spatio-temporal distribution of EGF-activated PKA in specific sites within the living cells. These data provide direct and in vivo evidence for PKA activation by EGF and add a new dimension for PKA signaling pathway.

关键词