

importin13 蛋白质介导 myopodin 蛋白质入核的研究

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核的研究

Nuclear Import of Myopodin Mediated by Importin 13

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摘要

生物大分子的核质转运是由受体 importin β 蛋白家族成员介导完成的。在研究胚胎肺发育的过程中,发现了一个新的 importin β 家族成员,命名为 importin13。Importin13 是目前所知的哺乳动物细胞中唯一具有双向转运功能的受体蛋白。另外研究还发现 Imp13 的表达受到糖皮质激素和机体发育期的双重调控,是目前所知的唯一一个受到双重表达调控的核质转运受体。

通过 Northern blot 分析发现, importin13 在胚胎肺、脑及心脏都有大量的表达,为了了解 imp13 在心脏中的功能,我们利用酵母双杂交系统在人的心脏 cDNA 文库中筛选可与 importin13 相互作用的蛋白质,筛选到 myopodin 蛋白(359-698aa)。据文献报道 myopodin 蛋白可能具有以下功能: 1. 与肌动蛋白结合形成结构蛋白; 2. 核质穿梭蛋白,参与信号转导; 3. 表达量的高低和核质分布与前列腺癌及膀胱癌的恶化有关,细胞核中 Myopodin 含量增加可以抑制前列腺癌及膀胱癌的转移和扩散。

虽然已有文献报道 importin α 和 14-3-3 蛋白介导 myopodin 蛋白的入核。但 importin α 本身不能单独介导蛋白质的入核,而是作为 importin β 家族成员的接头蛋白。因此有关 myopodin 如何进行核质转运的分子机理还未弄清,而 myopodin 在核质之间的转移与其抑制肿瘤的功能有很大关系。本论文研究了 myopodin 与 importin13 间的相互作用,并且探讨了由 importin13 介导的 myopodin 蛋白质的核质转运机制。

本研究发现:

1. 以 importin13 为诱饵蛋白在人心脏文库中通过酵母双杂交筛选到与之相互作用的 myopodin 蛋白,并通过 RT-PCR 从中国人骨骼肌中获得 MYOPODIN 全长 ORF 基因。
2. 通过 GST pull down 和免疫共沉淀实验验证了 importin13 与 myopodin 之间的相互作用。
3. 研究内源 myopodin 及其不同片段 myopodin 与 EYFP 融合蛋白(F-MPD, N-MPD, C-MPD, M-MPD)以及敲除 NLS 的 myopodin 突变体在不同细胞中的定位,发现

位于 N-端的 NLS1 无核定位功能，而 C-端的 NLS2 有较强的核定位功能，但还存在其他入核信号。

4. 异核体细胞融合证明 myopodin 是核质穿梭蛋白。
5. Importin13 是 myopodin 蛋白的入核转运受体：（1）myopodin 的 N 端不能与 importin13 相互作用，间接推测 importin13 识别 myopodin 的 C 端；（2）突变了 NLS1 和 NLS2 的 myopodin 依然能与 importin13 相互作用，说明还存在其他入核信号；（3）Ran 结合实验证明了 importin13 是 myopodin 的入核转运受体；（4）过量表达的 C-importin13 阻止内源 myopodin 的入核；（5）importin 7, importin 8, importin α 2, importin β 2 均不能与 myopodin 相互作用或作用力很弱，说明 importin 13 是 myopodin 的特异转运受体。

关键词：Importin13；Myopodin；核质转运

ABSTRACT

Nucleocytoplasmic transport of macromolecules is mediated by the soluble transport receptors collectively named importin β super family proteins. To investigate molecular mechanisms of lung organogenesis, we found a novel importin β homolog, named importin13. Importin13 is a novel mediator of nuclear import and export, the only bi-directional transporter in mammalian importins. Recent research reports described the demonstrated developmental and glucocorticoid regulation of importin 13 nucleocytoplasmic shuttling in fetal Lung, this dual regulative mechanisms was not found in other importin β membranes by now.

Importin13 was enriched in lung, brain, heart of fetal rat and human in Northern analysis. To investigate the function of importin13 in heart, we used importin13 protein as bait to screen a human normal heart cDNA library using the yeast two-hybrid system and found a cDNA containing the C-terminal 359-698 amino acids of human Myopodin. Recently some paper describe the characteristics of myopodin protein including: (1) structural protein (actin-bundling protein); (2) participating in a signaling pathway (nucleocytoplasmic shuttling between Z-disc and nucleus); (3) suppression of prostate and bladder cancer growth and metastasis. Most importantly, nuclear myopodin expression was a tumor suppressor.

An article explained the importin α binding and the subsequent nuclear import of myopodin are regulated by phosphorylation-dependent binding of myopodin to 14-3-3. However importin α proteins can not import into nucleus by themselves, they are transported by importin β as adaptor. Therefore the real nuclear import machinery of myopodin remains to be established. Because of the direct relationship between nucleocytoplasmic shuttle of myopodin and its tumor suppression activity, our research will focus the interaction of myopodin and importin13 and the nucleocytoplasmic transport mechanism of myopodin.

The results of these studies include:

1. We found myopodin was a novel imp13-binding protein by using the yeast two-hybrid system. To research the biological function of myopodin protein, we cloned the full length of myopodin open reading form gene from mRNA of Chinese

skeleton muscle by RT-PCR。

2. Interaction of full length myopodin and importin 13 was demonstrated *in vitro* by GST pulldown and *in vivo* by coimmunoprecipitation.
3. We observed the subcellular localization of endogenous myopodin protein、 recombinant protein containing different region of myopodin and YFP and myopodin NLS mutations. It was possible that myopodin had other NLSs besides NLS1 and NLS2, and the nuclear import activity of NLS1 was much weaker than NLS2.
4. Myopodin was proved to be a shuttling protein by heterokaryon nucleocytoplasmic shuttling assay.
5. Importin13 is the nuclear import receptor of myopodin: (1) N-myopodin can not interact with importin13, which indirectly approved that importin13 binded to C-myopodin; (2) The mutation of two potential NLSs did not affect the interaction of myopodin mutation and importin13, it was possible there were other no-classical NLSs in myopodin protein; (3) Results of Ran binding assay showed that importin13 was nuclear transport receptor of myopodin; (4) Nuclear entry of endogenous myopodin is blocked by the C-terminal IPO13; (5) There were no or weak interaction between myopodin and importin7, importin8, importin α 2, importin β 2 respectively, which confirmed that importin13 was the especial nuclear transportor of myopodin.

Key words:importin13; myopodin; nucleocytoplasmic transport

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