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PDF文档

人口腔癌症缺失相关蛋白（DOC-1R）的表达、纯化和晶体生长

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口腔癌缺失(Deleted in Oral Cancer-1, DOC-1)基因是近年来被证实的口腔癌中口腔癌缺失（Deleted in Oral Cancer-1, DOC-1）基因是近年来被证实的口腔癌中具有抑癌作用的基因。1999年，研究人员通过酵母双杂交实验又发现了与DOC-1相关的另一候选抑癌基因DOC-1R（DOC-1 related）。以往的很多实验表明，这两个蛋白无论序列还是功能上都非常相似。然而，其三维结构以及与其他重要蛋白相互作用的机制一直还不清楚，PDB库中也未见其相关同源结构的报道。作者将人DOC-1R基因的cDNA片段克隆至原核表达载体pET-22b(+)中，通过IPTG诱导获得高效表达，再经过Ni-NTA亲和层析和Superdex 75层析柱纯化，获得了纯度达到96% 以上的蛋白。质谱分子量测定显示DOC-1R的分子量为14091.23 Da，与理论分子量基本一致；动态光散射实验显示蛋白均一性高达99.0%，可用于晶体生长；采用悬滴气相扩散法筛选，在多个条件下得到了DOC-1R的微晶。为DOC-1R的三维结构解析奠定了坚实的基础。

Expression, purification and crystallization of human DOC-1R

DOC-1(Deleted in Oral Cancer-1) is a growth suppressor gene identified first in oral cancer. DOC-1R(DOC-1 related) gene is another tumor suppressor gene which was cloned in 1999. Recent studies suggested that DOC-1 and DOC-1R share similarity in both of their sequences and functions. However, there is no homology structure found in PDB. Human doc-1r gene was cloned into expression vector pET-22b(+) and over-expressed in E.coli strain BL21 (DE3). Human DOC-1R protein was purified in a two-step procedure of Ni-NTA affinity followed by Superdex 75 chromatography. Over 98% of purified DOC-1R proteins was finally got for structural and functional research. Mass Spectrum identified that the exact molecular weight of DOC-1R is 14091.23 Da; Dynamic light scattering assay showed that its homogenesis was over 99.0%. The crystals of DOC-1R were obtained by using hanging-drop vapor-diffusion method.

关键词