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论著

反式激活蛋白-氧依赖性降解结构融合结构域介导蛋白跨膜转运和氧依赖性降解作用

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摘要 **目的** 研究反式激活蛋白(TAT)蛋白转导结构域介导的融合蛋白的跨膜转运，以及氧依赖性降解(ODD)结构域介导融合蛋白在体内外不同氧分压环境中的降解作用。**方法** 将TAT，ODD以及增强绿色荧光蛋白(EGFP)基因序列进行融合，构建了TAT-ODD-EGFP融合蛋白基因。将该序列克隆到原核表达载体pET28a中，在BL21工程菌中进行表达，通过亲和柱层析法纯化，得到高纯度的融合蛋白。同方法制备EGFP和TAT-EGFP融合蛋白作为对照。在20%O_2和1%O_2将融合蛋白与A549，H1299和MDA-MB-231细胞系孵育，荧光显微镜下观察融合蛋白在细胞中的分布以及稳定性。小鼠尾静脉注射不同融合蛋白 (10 mg·kg^{-1})，分别在注射后1，6和12 h处死动物，取脏器在荧光显微镜下观察融合蛋白的分布及稳定性。**结果** 由于EGFP相对分子质量大，无法穿透细胞及组织。TAT可以有效地介导TAT-EGFP进入细胞和动物实体组织，其稳定性不受细胞或组织中氧分压的影响，而TAT-ODD-EGFP进入细胞或组织后，在20%O_2条件下迅速降解，但可稳定存在于1%O_2细胞及组织中。**结论** TAT可以有效地转导融合蛋白穿过细胞膜。引入ODD，20%O_2下融合蛋白TAT-ODD-EGFP迅速降解，但可以稳定存在于1%O_2细胞和组织中，说明TAT-ODD可以转导蛋白进入并靶向存在于低氧的肿瘤组织中。

关键词 低氧 依赖性降解结构 TAT 增强型绿色荧光蛋白

分类号 Q71

Transmembrane delivery and oxygen-dependent degradation mediated by trans- activator-oxygen-dependent degradation fused motif

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Abstract

OBJECTIVE To evaluate transmembrane activity of trans-activator (TAT) protein transduction domain and effect of oxygen-dependent degradation (ODD) domain under different oxygen tension microenvironments *in vitro* and *in vivo*. **METHODS** An enhanced green fluorescent protein (EGFP) fusion protein conjugate with TAT and ODD was constructed, expressed and purified via a series of molecular biological procedures. EGFP and TAT-EGFP were also prepared following similar manipulation. To assay the cell-permeability and hypoxia-targeting stability *in vitro*, various tumor cell lines including H1299, A549 and MDA-MB-231 were cultured with different EGFP fusion proteins under 20% and 1% oxygen tension conditions, respectively. The treated cells were fixed and then directly observed by fluorescence microscope. To evaluate the oxygen-dependent stability and the profile of distribution of TAT-ODD-EGFP *in vivo*, male BALB/c mice were iv given EGFP, TAT-EGFP and TAT-ODD-EGFP 10 mg·kg^{-1}. Animals were executed to harvest organs, including the heart, liver, spleen, lung, kidney, intestine, and brain at 1, 6 and 12 h after treatment. The frozen sections were prepared for assay by fluorescence microscope. **RESULTS** TAT-EGFP could be effectively delivered into and stabilized in the different cell lines *in vitro* and all tissues of mice were observed *in vivo*. Like TAT-EGFP, TAT-ODD-EGFP could be transformed into cells by TAT, but its stability was oxygen-dependent because of its degradation property from ODD under different oxygen tensions. **CONCLUSION** AT can deliver its fusion proteins into cells and animal tissues effectively. TAT fusion protein conjugates with ODD was stabilized in hypoxic cells and tissues, but it was degrades quickly in normoxia because of ODD′s function. TAT-ODD domain can be used in the targeted transduction of anti- tumor proteins into hypoxic tumor tissues.

Key words hypoxia oxygen-dependent degradation domain trans activator enhanced green fluorescent protein

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