

针对核质运输的高通量小分子筛选方法

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摘要 核质运输是真核细胞的重大基本生命活动。核质运输小分子抑制剂不仅可以广泛应用并促进核质运输相关的基础研究,同时也为相关疾病、尤其是病毒性疾病的药物开发提供有利线索。然而,目前针对核质运输的商业化小分子仅有Leptomycin B一种。建立一个针对整个核质运输通路的小分子筛选平台,将有利于筛选与获得多种干扰核质运输的小分子。文章利用NZGFP和CZGFP可以重组为具有荧光GFP的特性,构建NZGFP-NES和CZGFP-NLS,将NZGFP和CZGFP分别定位在细胞质与细胞核中;当核内运或核外运通路被干扰,NZGFP和CZGFP定位发生改变并聚集重组为具有荧光的GFP。该方法可以有效检测核外运小分子抑制剂Leptomycin B的作用,为针对整个核质运输通路的高通量小分子筛选提供了一个有效平台。

关键词: 核质运输 高通量筛选方法 NZGFP/CZGFP Leptomycin B

Abstract: Nuclear transport, an essential cellular process of eukaryotic cells, plays critical roles in differentiation, development, as well as viral disease and oncogenesis, making the small molecules that target this process very intriguing not only in fundamental research but also in disease treatment. However, only one compound, Leptomycin B, is commercially available to inhibit nuclear transport. Therefore, it will be a great advantage to establish an assay that targets the whole nuclear transport pathway for screening and obtaining small molecule that regulates nuclear transport. In this study, we established an efficient nuclear transport assay based on the reconstitution of GFP by NZGFP and CZGFP. We constructed NZGFP-NES and CZGFP-NLS, making them locate in cytoplasm and nucleus separately. Their distribution will be changed when nuclear transport is interfered, resulting in co-localization of NZGFP-NES and CZGFP-NLS and subsequent reconstitution of fluorescent GFP. The inhibiting effect of Leptomycin B on nuclear transport can be sensitively detected by NZGFP-NES/CZGFP-NLS report system, providing an efficient assay for high-throughput screening of small molecule against nuclear transport.

Keywords: nuclear transport, high-throughput screening assay, NZGFP/CZGFP, Leptomycin B

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