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短寡核苷酸链高效转染体外培养恶性疟原虫的研究

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Evaluation of Transfection Effectiveness Using Fluorescein-labelled Oligonucleotides and Entraster-R siRNA Transfection into *Plasmodium falciparum*

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摘要 5%山梨醇连续2次同步化恶性疟原虫培养物（8 h窗口），培养16 h后，直接孵育组（A组）将50 μl含寡核苷酸培养基与450 μl恶性疟原虫培养物（5%虫血率，1%血压积）混合孵育，Entranster-R试剂转染组（B组）将50 μl转染复合物（含寡核苷酸链和转染试剂）与450 μl恶性疟原虫培养物混合孵育，培养5 h后重悬，分别取出250 μl，1 500×g离心3 min，收集沉淀，进行荧光显微镜观察和流式细胞术检测转染效率。剩余细胞经RPMI 1640培养基洗涤1次后，加入500 μl含2%新鲜红细胞的培养基，继续培养12 h至第2个细胞周期，再次进行流式细胞术检测。荧光显微镜观察结果显示，Entranster-R试剂转染组可明显观察到感染红细胞中标记探针的绿色荧光，而直接孵育组未观察到绿色荧光。流式细胞术检测结果表明，Entranster-R试剂转染组小分子寡核苷酸转染疟原虫的效率可达（47.40±3.39）%，高于普通孵育法［（0.60±0.27）%］，且该组在第2个周期中维持转染率约（26.85±2.90）%，而直接孵育组在第2个细胞周期则几乎检测不到。提示利用纳米转染试剂Entranster-R能提高寡核苷酸转染疟原虫的效率。

关键词： 恶性疟原虫 Entranster-R转染 寡核苷酸链

Abstract： The cultured *Plasmodium falciparum* parasites were synchronized twice by 5% sorbitol treatment twice（8-hour window）, and then incubated at 37 ℃ for 16 h. Parasites were transfected with fluorescein-labelled oligonucleotides （group A） or fluorescein-labelled oligonucleotides+Entranster-R siRNA transfection reagent （group B）. After 5 h a part of parasites was evaluated by fluorescence microscopy and flow cytometry. The rest of parasites were washed with RPMI 1640 medium, and then incubated with 500 μl new medium containing 2% fresh erythrocytes for another 12 h, and detected by flow cytometry. The fluorescein-labelled oligonucleotides were localized in erythrocytes in group B, but nearly no fluorescence was observed for group A. Flow cytometry analysis indicated that the transfection efficiency of group B [（47.40±3.39）%] was higher than that of group A [（0.60±0.27）%]. In the second cell cycle, the transfection efficiency in group B was （26.85±2.90）%, while that of group A was nearly zero. The results indicated that Entranster-R siRNA transfection reagent may increase the oligonucleotides transfection efficiciency.

Keywords： *Plasmodium falciparum* Transfection Oligonucleotides

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