利用 polyethylenimine 包覆 antisense oligonucleotide 的運輸系統之研究

Study of polyethylenimine-based antisense oligonucleotide delivery system

中文摘要

癌細胞與正常的細胞最大的差別在於癌細胞的細胞凋零受到了抑制，使得癌細胞可以不正常的增生。細胞凋零的過程，受許多蛋白質之間的平衡所調控。Bcl-2 為其中一個與細胞凋零息息相關的蛋白質，在一些癌細胞中，因為 Bcl-2 的過度增生而抑制了細胞凋零的發生，造成癌細胞不正常的增生。有許多方法可用來降低 Bcl-2 在癌細胞中之表達，以間接達到增加細胞凋零的目的，例如運送 Bcl-2 antisense oligonucleotide (G3139)至標的細胞，然而反譯寡核苷酸(antisense oligonucleotides, ODNs)在體內運送過程中易遭許多酵素及其他大分子物質破壞，因此本論文將探討利用聚合物包覆 G3139 以達到有效率運送反譯寡核苷酸的目的。有些癌細胞像是 K562 和 NCI-H929，細胞膜有運鐵蛋白接受體(transferrin receptor ,TfR)過度表現，因此我們可以藉由運鐵蛋白(transferrin ,Tf)來進行標靶運輸 (targeted delivery)，以增加藥物進入細胞內的量。

於本論文中，我們利用西方墨點法來測定細胞內蛋白質的多寡，以了解所給予的反譯寡核苷酸是否有發揮作用。並且製備 polyethylenimine- oligonucleotide/FITC (PEI-ODN/FITC)複合物，將此複合物給予細胞，利用螢光顯微鏡及流式細胞儀來觀察此複合物在細胞內的分布情形及進入細胞內的量。Desferrioxamine (DFO) 可以增加細胞膜表面運鐵蛋白接受體的表現，因此可以藉由給予 DFO 來增加癌細胞表面運鐵蛋白接受體的表現量，進一步增加藥物複合物進入癌細胞的量，產生療效加成作用。

從目前的結果，的確在癌細胞中含有 Bcl-2。另外，從流式細胞儀和螢光顯微鏡的結果，可以發現 PEI-ODN/FITC 複合物進入細胞內的量，會受到一開始所給予細胞的 PEI-ODN/FITC 複合物量的不同而有差異，也會因為複合物的 N/P ratio 不同而有所影響。當 PEI-ODN 複合物的 N/P ratio 越高，對細胞的毒性也越大。然而，到目前為止的西方墨點法之結果，仍然無法看出有無投與 G3139 進入細胞之間的差異，這部分的實驗有待改進。

英文摘要

One of the major differences between cancer cells and normal cells is the inhibited apoptosis of cancer cells. Apoptosis is regulated by the balance between many proteins during this process. Among them, Bcl-2 is one of the most important inhibitory proteins in the pathway. Many cancer cells proliferate abnormally due to

overexpression of Bcl-2. Various strategies were developed to downregulate the Bcl-2 expression, which indirectly achieve the aim of increasing apoptosis. For example, delivering Bcl-2 antisense oligonucleotide (G3139) to targeting cells is an attractive strategy. However, antisense oligonucleotides (ODNs) is easily destroyed by enzymes, nucleases, and other macromolecules in vivo. Therefore, in this study, we will develop and evaluate polymers containing ODNs. The aim of the study is to deliver the ODNs to targeting cells efficiently and selectively.

Delivery of G3139 was evaluated by measuring the amount of Bcl-2 protein western blot. Cellular uptake of polyethylenimine- oligonucleotide/FITC (PEI-ODN/FITC) complex was observed by flow cytometry and fluorescence microscope. In order to enhance the targeting effect, cells were treated with desferrioxamine (DFO), which reportedly can up-regulate the expression of TfR located on cell membrane.

The current data showed that Bcl-2 exists in cancer cells used in this study. From the data of flow cytometry and fluorescence microscope, we found that the amount of PEI-ODN/FITC entering cells corresponds with the initial amount of PEI-ODN/FITC and the N/P ratio of PEI-ODN/FITC. As the N/P ratio increasing, the cytotoxicity of complex raised. From the western blot data until now, unfortunately there were not significant differences between G3139 treating or not treating. Therefore, something should be improved in the study..