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一、中文摘要

在熱帶和亞熱帶地區許多人在鼻側之眼輪部長一合併表皮細胞病變的纖維血管組織，稱為翳狀贅肉o 而此疾病據流行病學和光學的研究和紫外線的直接照射在眼輪部有相當大的關係o 翳狀贅肉在一般組織病理切片中可見纖維母細胞有增生的現象、而其上皮細胞則可見細胞層數變少呈現萎縮。而在以前體外細胞實驗中發現眼睛翳狀贅肉纖維母細胞呈現半轉形之狀態(semi-transformation)，細胞對生長因子和血清之依賴減少，在洋菜膠之表面可形成聚落，細胞的生長速度也變快了，但是在裸鼠中並未形成明顯之腫瘤等現象。而最近的文獻中也可發現紫外線(UVB)可以藉細胞表面的生長因子接受體的聚合作用(EGF，IL-1，TNF)和內在化來活化細胞內之c-Jun活化酵素，進而活化細胞內之轉錄因子而使細胞內之表現形改變。而細胞半轉形化有可能因為這種因素而造成。在轉形細胞中可發現許多基因被活化，其中一項就是細胞之telomerase被活化，telomere位於細胞內染色體之尾端可以保護染色體之正長結構。正常細胞之壽命是有限也就是正常細胞在體外培養時其繁殖世代是有限的，其telomere的長度會隨細胞的老化而逐漸縮短，但是在一些癌細胞或轉形細胞中其telomerase被活化而造成染色體的尾端telomere不會隨著細胞的的增殖而縮短。本研究計劃之目的就是假設眼睛翳狀贅肉纖維母細胞在接受紫外線照射之後產生半轉形化，而要證明細胞之半轉形化除了上面的方法之外，對其telomerase activity之測定也是另外一種方式，本研究計劃在體外的實驗下測定翳狀贅肉纖維母細胞和結膜纖維母細胞telomerase activity藉以證實翳狀贅肉纖維母細胞被紫外線活化而半轉形化。
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關鍵詞：專題計畫、報告格式、國科會

Abstract

Pterygium is a degenerative corneal limbal process characterized by the triangular conjunctival invasion onto the cornea (1,2). The histopathological pictures include an atrophic conjunctival epithelium and a highly vascularized bulky mass of hypertrophic and elastotic degenerated connective tissue (3). On the corneal side under the head of pterygium, fibroblasts penetrate Bowman's layer basement membrane. The invasive characteristics of pterygial fibroblasts seem to like the transformed cells such as tumor cells(4-10). In the previous study pterygial fibroblasts showed the phenotype of semitransformed cells, including lower serum requirement、higher saturation cell density、lower requirement of exogenous growth factors and colony formation in soft agar culture、digestion of the basement membrane of epithelium, but no tumor formation after pterygial fibroblast subcutaneous injection in nude mice (11). The epidemiological and mathematical studies showed that the formation pterygium was correlated with the UV irradiation (12,13). Whether the semitransformed characteristics of pterygial fibroblasts are due to ultraviolet irradiation is highly speculated.

Telomeres, composed of TTAGGG repeats, have been shown to shorten as a function of age in vivo and during the passage of normal cells in vitro (14-20). The loss of telomere sequence is thought to contribute to senescence(20-21). A variety of human and other animal cell types can now be grown in the laboratory, and the majority of these cell cultures have a finite ability to proliferate. After accruing a number of population doublings, they enter the terminally nondividing state referred to as replicative senescence. The first evidence that telomere loss occurred during aging was from an analysis of cultured human fibroblasts, in which the mean length of the terminal restriction fragment was found to decrease in a replication-dependent manner (19,21). Activation of mechanism to restore telomeres, namely, telomerase activation, has been proposed to be critical event in the immortalization of human cells (14,22-24). These cells express the enzyme telomerase, which adds essential telomerase sequences to maintain the ends of chromosomes. Such results led to the proposal that during successive rounds of DNA replication, progressive loss of telomere length until a critically short telomere length is sensed as DNA damage and causes cells to exit the cell cycle. Immotal cells need a mechanism to stablize chromosome ends and therefore expression of telomerase is required for immortalization. However, additional studies of telomeres and telomerase in cell senecescence have revealed a more complex picture than that proposed in the hypothesis. Telomerase-negative cell lines have been identified, and treatment of immortal human B and T cell lines with inhibitors of reverse transcriptase reduces telomerase activity, with no effect on the immortal phenotype (25-28). On the other hand, some somatic cells have been found to express telomerase, although their telomere continue to shorten with rounds of replication (29). These data suggest that telomerase activity alone does not maintain telomere. They also indicate that telomerase-independent mechanisms to stabilize chromosome ends must exist. Nonetheless, the telomere hypothesisis presently the most viable hypothesis as a molecular basis for a mitotic clock.

In a recent study which revealed that exposure of mammalian cells to ultraviolet light activates the c-Jun amino-terminal protein kinase (JNK) cascade, causing induction of many target genes (30). Exposure to UV light induced clustering and internalization of cell surface receptors for epidermal growth factor (EGF), tumor necrosis factor (TNF), and interleukin-1 (IL-1). Whereas activation of each receptor of each receptor alone resulted in modest activation of JNK, coadministration of EGF, IL-1, and TNF resulted in a strong synergistic response equal to that caused to UV light. These results suggest that UV might lead a different pathway of preventing senescence.

From the above discussion we suspect that semitransformed pterygial fibrblasts from chronic UV irradiation are activated through some pathways such as

internalization and activation of growth factor receptors, then activate JNK and telomerase. Thus, we design the following experiments for testing our hypothesis.

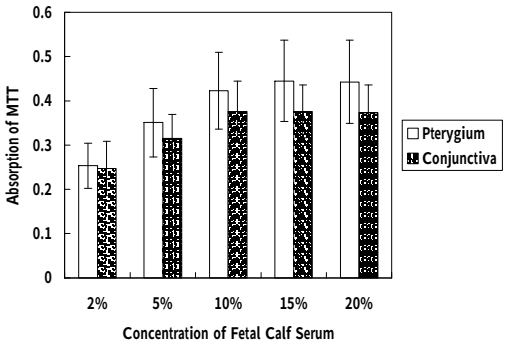
Polymerase chain reaction-based telomerase repeat amplification protocol (PCR-based TRAP) assay is designated to increase the sensitivity, speed, and efficiency of detecting telomerase reaction (31). In the TRAY assay, telomerase synthesizes extension products, which then serve as the templates for PCR amplification. Reaction conditions suitable for both telomerase and Tag polymerase activities are allowed to be performed in a single reaction tube.

This project is composed of three part; first, we use TRAP assay to compare the telomerase activity of pterygial fibroblasts with normal conjunctival fibroblasts; second, the proliferative activity will be compared using the MTT assays ^{3}H-Thymidine incoperation test; third, we test the telomerase activity directly from the surgical specimens, then correlate the recurrence rate after certain kind of surgical procedures with the activity of telomerase or proliferation.

In clinical part, we divided the clinical presentations of pterygium into three kinds of manifestations; atrophic, intermediate and inflammatory. The atrophic type is defined that the scleral vessels can be seen through the pterygial body, the inflammatory type is defined as injected vessels in pterygium and scleral vessels can not be seen and intermediate type is manifested between above two types. We use bare sclera with a one-minute of 0.02% mitomycin application to excise the pterygium in these three groups. Then, the surgical specimens will be tested for telomerase and proliferative activity and correlate with the recurrence rate 6 months and 12 months later..

三、結果與討論

W first used MTT assay to determine the proliferative abilities between normal conjunctival and pterygial early-passage fibroblasts. We found pterygial fibroblasts are more proliferative than conjunctival fibroblasts under different concentration of fetal calf serum (figure below).



But when we used the PCR-based TRAP ELISA test to differentiate the activities of telomerase between pterygial and conjunctival fibroblasts, we could find any difference between two kinds of cells under different concentration of fetal cal serum. The telomerase activities did not activate in these cells.

Therefore, we concluded that the pterygial fibroblasts were not transformed tumor cells. They are activated cells, either by inflammatory cytokines or UV irradiation.

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