行政院國家科學委員會專題研究計畫成果報告

介白質-1 對牙齦纖維母細胞老化之影響及其在分子及細胞學上之作用機轉

Molecular and cellular mechanisms of IL-1 effect on cellular aging in human periodontal fibroblasts

計畫編號：NSC 88-2314-B-038-136
執行期限：87 年 8 月 01 日至 88 年 7 月 31 日
主持人：周幸華
執行機構及單位名稱:台北醫學院牙醫學系

一、中文摘要

牙周軟組織之大部分是由人類牙周纖維母細胞所構成. 牙周纖維母細胞具有維持牙周結締組織之完整性及功能性的作用. 體外培養之纖維母細胞因為有一定之壽命, 長久以來已被研究者設定為研究細胞老化之實驗模型. 近年來, 有研究發現高齡者由來之低繼代纖維母細胞及年輕者由來之高繼代纖維母細胞均會自發性地分泌介白質-1 (Interleukin-1), 因而誘導膠原蛋白胨、stromyelysin、及錳超氧化物歧化胨等基因之表現. 另有報告指出這三種基因表現亦可能與細胞之衰老(senescence)有關.

本計畫的研究目的是探討在體外培養之牙周纖維母細胞在老化過程中所分泌之介白質-1 對其本身之增生及衰老的影響, 並研究其在分子及細胞學上之作用機轉. 本計畫分為三部分進行. 首先, 利用基因導入的方法將第二型之介白質-1 接受器過剩表現於細胞上來拮抗介白質-1 對細胞之作用. 其次, 我們比較未轉形細胞、轉形細胞、及過剩表現轉形細胞之增生. 最後, 我們分析熱休克蛋白 27 及與細胞衰老有關的三種基因(膠原蛋白胨、stromyelysin、及錳超氧化物歧化胨)的表現情形來探討介白質-1 對細胞衰老之作用機轉.

本研究的結果發現隨著細胞老化, 高繼代纖維母細胞之錳超氧化物歧化 及 stromyelysin 基因之表現比低繼代纖維母細胞之基因表現為強. 比較轉形細胞及第二型介白質-1 接受器過剩表現轉形細胞之增生,發現第二型介白質-1 接受器過剩表現轉形細胞之增生受到抑制. 第二型介白質-1 接受器過剩表現轉形細胞之熱休克蛋白 27 的表現雖較轉形細胞之表現為高, 但是兩群細胞與細胞衰老有關的三種基因(膠原蛋白胨、stromyelysin、及錳超氧化物歧化胨)的表現情形並無顯著之差異. 第二型介白質-1 接受器過剩表現轉形細胞之增生受到抑制之作用機轉仍需進一步之研究來解明.

關鍵詞：牙齦纖維母細胞, 介白質-1, 介白質-1 接受器, 細胞衰老

Abstract

Human diploid fibroblasts (HDFs) are the major components of gingival connective tissues and play pivotal roles in maintaining the functional integrity of gingival tissues. The lifespan of HDFs before senescence *in vitro* is inversely proportional to the age of the donor *in vivo*, suggesting that cellular senescence *in vitro* reflect aging *in vivo*. Recently, age-associated increases in production of Interleukin-1 (IL-1; IL-1α and IL-1β) have been reported to be correlated with overexpression of IL-1-induced genes in late-passage HDFs obtained from young tissue donors and in early-passage of HDFs obtained from old tissue donors. These IL-1-induced genes including collagenase, stromyelysin and a scarvenger enzyme of anion, manganese superoxide dismutase (Mn SOD).

Though, there are increasing evidences of the biological significance of age-associated IL-1 in HDFs *in vitro*, there is little known about the underlying

mechanisms of its effects. The goal of this grant application is to characterize the roles of age-associated IL-1β in the regulation of biological aging and cellular proliferation in HGFs, and to understand the underlying molecular mechanisms. To do this, we analyzed the production of IL-1β and in young- and late-passage cells of non-transformed HGFs, vector-transfected HGFs and RII-transfected HGFs. Then, we compared the proliferation rate of cell in these HGFs. Finally, to investigate the molecular mechanisms of the effects of age-associated IL-1β on HGFs, we examined the expression of hsp 27 and the senescence-related genes in these HGFs by reverse transcriptase- polymerase chain reaction (RT-PCR).

The data showed a decreased proliferation rate of the RII-transfected GF than that of the vector-transfected GF. As the cell grew older, an increased gene expression of MnSOD and stromyelysin were detected in late-passage GF. Although there was no significant difference in the expression of these genes in RII-transfected GF and the vector-transfected GF, an increased basal level of hsp 27 in RII-transfected GF was detected. These results suggest that IL-1RII overexpressing on GF might play a role in regulation of cell proliferation, but the underlying mechanisms of this regulation need to be further investigated.

Keywords: type II Interleukin-1 receptor, aging fibroblasts, magnaese superoxide dismutase

二、緣由與目的

Age is considered as a correlate rather than a risk factor of periodontal disease (1). Prevalence of clinical loss of attachment and gingival recession increased with age (2). Age was also positively associated with alveolar bone loss in periodontal patients (3). However, the question of why periodontal disease is more severe in elderly people is not completely answered and is still open for further investigations.

Human diploid fibroblasts (HDFs) are major components of gingival connective tissues and play pivotal roles in maintaining the functional integrity of gingival tissues. HDFs have a limited capacity to proliferate in culture (4, 5). After 20-60 population doublings, HDFs undergo a process called cellular senescence. The number of cell divisions before the onset of senescence is inversely correlated with the age of tissue donors, suggesting that cellular senescence *in vitro* reflect aging *in vivo* (4,5). Because of their limited proliferation, HDFs have been used as a model for *in vitro* study of aging (6).

Recently, age-associated increases in production of Interleukin-1 (IL-1; IL-1α and IL-1β) have been reported to be correlated with overexpression of IL-1-induced genes in late-passage HDFs obtained from young tissue donors (7-9). Overexpression of these IL-1-induced genes including collagenase, stromyelysin and a scarvenger enzyme of anion, manganese superoxide dismutase (Mn SOD), were also found in early-passage of HDFs obtained from old tissue donors (8,10). The reports from *in vivo or in vitro* studies demonstrated that aging is associated with changes in expression of genes that are involved in inflammation and connective tissue remodeling. Overexpression of these genes in late-passage fibroblasts might change the phenotype of these cells during aging and play roles in the decline of biological functions in aging human fibroblasts.

While there are increasing evidences of the biological significance of age-associated IL-1 in aging human fibroblasts *in vitro*, there is little known about the underlying mechanisms of its effects.

Since there is little information available on the age-associated IL-1β in the regulation of biological aging and cellular proliferation in human gingival fibroblasts (HGFs), the present study was designed to investigate the effects of age-associated IL-1β on the cellular proliferation, and the underlying molecular mechanisms. To do this, we analyzed the production of IL-1β in

young- and late-passage cells of non-transformed HGFs, vector- transfected HGFs and type II IL-1R overexpression HGFs (RII-transfected GF). Then, by using overexpression of type II IL-1R to antagonize the effects of age-associated IL-1β, we compared the cellular proliferation in these HGFs. Finally, to investigate the molecular mechanisms of the effects of age-associated IL-1β on HGFs, expression of hsp 27, which is the early event of IL-1 signal transduction and might be related to cellular proliferation (11), was analyzed by immunoblot. Also, expression of the senescence-related genes in these HGFs was analyzed by reverse transcriptase- polymerase chain reaction (RT-PCR).

三、結果與討論

In the present study, all experiments were done on one strain of 3 separated clones of each non-transfected, vector-transfected, and RII-transfected HGFs.

We first overexpressed type II IL-1R to antagonize the effects of age-associated IL-1 on HGFs. HGFs were obtained from biopsies of the attached gingiva of a 19-years old volunteer with clinically and radiographically normal periodontal tissues. HGFs were transfected, selected and cloned as previously described (12-14). Positive clones with type II IL-1R overexpression were screened by RT-PCR at mRNA level. Type II IL-1R overexpression at protein level was confirmed by immunostaining with a monoclonal anti-human type II IL-1R antibody.

After the establishment of positive clones of RII-transfected GF, the production of age-associated IL-1β increases in late-passage HGFs were determined using enzyme-linked immunosorbent assay (ELISA) kits purchased from R & D system. The data showed that the levels of age-associated IL-1 increased in the late-passage HGFs.

Comparisons of the effects of age-associated IL-1 on the proliferation of HGFs in vitro were performed by utilizing [^{3}H] thymidine incorporation and cell counting in a Coulter counter. The data showed that RII-transfected GF showed a decreased cellular proliferation than vector-transfected GF (figure 1). However, the data of [^{3}H] thymidine incorporation showed no significant difference between RII-transfected GF and vector-transfected GF without stimulation. The effect of IL-1β on the inhibition of [^{3}H] thymidine incorporation was more prominent in vector-transfected GF than RII-transfected GF (figure 2).

To investigate the underlying mechanisms of age-associated IL-1 effected changes of the cellular proliferation of HGFs *in vitro*. We analyzed the expression of IL-1β-induced genes that is senescent-related by reverse transcriptase- polymerase chain reaction (RT-PCR).

Increased gene expression of MnSOD and stromyelysin were detected in late-passage GF (figure 3). There is no significant difference between the gene expression in RII-transfected GF and Vector-transfected GF. However, in the RII-transfected GF, an increased basal level of hsp 27 expression was detected.

Theses results suggest that IL-1RII overexpressing on GF might play a role in regulation of cell proliferation, but the underlying mechanisms of this regulation need to be further investigated.

四、計畫成果自評

於本研究計畫中，我們已完成下列之預期工作項目:

1. 利用基因導入的方法建立第二型介白質-1 接受器過剩表現之轉形牙周纖維母細胞株.
2. 藉著第二型介白質-1 接受器過剩表現來拮抗介白質-1 對細胞本身之作用，來檢討牙周纖維母細胞在老化過程中所分泌之介白質-1 對其本身之增生及衰老的影響.
3. 由檢查細胞內與細胞增生及衰老之訊息傳遞有關的因子、熱休克蛋白 27 及與細胞衰老有關的三種基因(膠原蛋白腖、stromyelysin、及錳超氧化物歧化腖)的表現情形、來探討介白質-1 對細胞增生及老

化之作用機轉.

本計畫之研究成果可以幫助瞭解牙周纖維母細胞在細胞老化過程中所分泌之介白質-1 於牙周結締組織中對細胞之增生及衰老之影響，及其在分子及細胞學上之作用機轉，並間接幫助了解介白質-1 對老化細胞之發炎反應及對細胞外間質之破壞與修復之影響.

五、參考文獻

1. Abdellatif HM, and Burt BA. An epidemiological investigation into the relative importance of age and oral hygiene status as determinants of periodontitis. J. Dent. Res. 1987; 66(1): 13-18.
2. Brown LJ, Brunelle JA, and Kingman A. Periodontal status in the united states, 1988-91: prevalence, extent, and demographic variation. J. Dent. Res. 1996; 75 (Spec Iss): 672-683.
3. Grossi SG, Genco RJ, Machtei EE, HO Aw, koch G, Dunford R, Zambon JJ. Assessment of risk indicators for alveolar bone loss. J. Periodontol. 1995; 66:23-29.
4. Hayflick L, The limited *in vitro* lifetime of human diploid cell strains Exp. Cell Res. 1965; 37:614-636.
5. Hayflick L. and Moorhead PS. The serial cultivation of human diploid cell strains. Exp. Cell Res. 1961; 25:585-621.
6. Goldstein S. Replicative senescence: Human fibroblasts come of age. Science. 1990; 249:1129-1133.
7. Kumar S, Millis AJT, and Baglioni C. Expression of interleukin 1-inducible genes and production of interleukin 1 by aging human fibroblasts. Proc. Natl. Acad. Sci. USA. 1992; 89:4683-4687.
8. Kumar S, Vinci JM, Millis AJT, Baglioni C. Expression of interleukin-1α and β in early passage fibroblasts from aging individuals. Exp. Geronto. 1993; 28: 505-513.
9. Zeng G, Millis AJT. Differential regulation of collagenase and stromelysin mRNA in late passage cultures of human fibroblasts. Exp. Cell Res. 1996; 222:150-156.
10. Allen RG, Keogh BP, Gerhard GS, Pignolo R, Horton J, and Cristofalo VJ. Expression and regulation of superoxide dismutase activity in human skin fibroblasts from donors of different ages. J. Cell Physiol. 1995; 165:576-587.
11. Maier JAM, Voulalas P, Roeder D, and Maciag T. Extension of the life span of human endothelial cells by an Interleukin-1a antisense oligomer. Science. 1990; 249: 1570-1574.
12. Chou HH. Roles of Type II interleukin-1 receptor expressed on human gingival fibroblasts in cytokine network. J. Okay. Dent. Soci.1997, 15 (1): 71-84.
13. Chou HH, Takashiba S, Takigawa M, Nishimura F, Arai. H and Murayama y. Role of Interleukin-1 receptors expressed on human gingival fibroblasts. J. Dent. Res. 1996; 75 (Spec Iss): 97 (635).
14. Chou HH, Takashiba S, Takigawa M, Nishimura F, Arai. H and Murayama y. Roles of type II Interleukin-1 receptor expressed on human gingival fibroblasts. J. Dent. Res. 1997; 76 (Spec Iss): 406 (3137).

本研究之部份結果預定於下列學會發表：

78th General Session & Exhibition of the International Association for Dental Research (2000, April, Washinton D.C.): Proliferation of gingival fibroblasts was inhibited by type II Interleukin-1 receptor gene transfer.

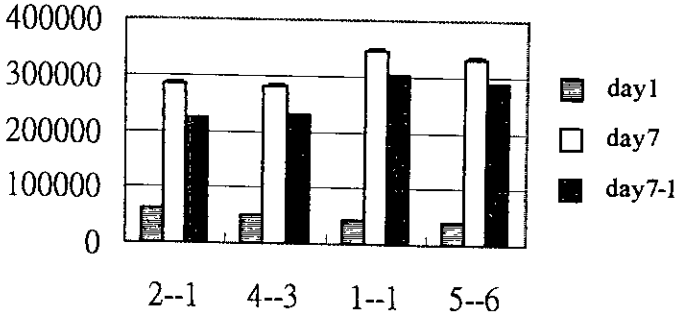

Fig.1. Compare cellular proliferation by cell counting in Coulter counter. RII-transfected GF (clone 2-1, 4-3) and Vector-transfected GF (clone 1-1, 5-6) were plated in 6-well plate at a density of 40000 cells per well. After cells were seeded, cells were trypsinized and counted in Coulter counter at indicated time. (Mean ± S.E.; * $p<0.05$)

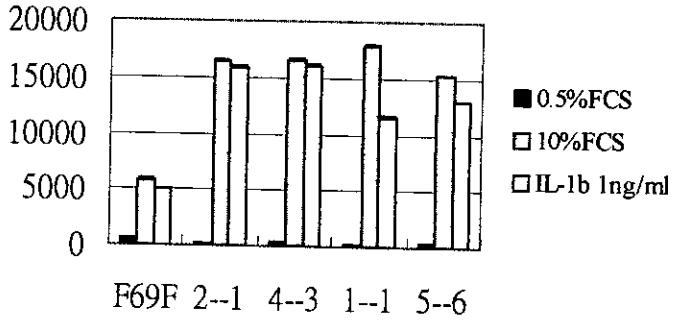

Fig.2. Compare cellular proliferation by utilizing [^{3}H] thymidine incorporation. Non-transfected GF(F69F), RII-transfected GF (clone 2-1, 4-3) and Vector-transfected GF (clone 1-1, 5-6) were plated in 24-well plate at a density of 20000 cells per well. After cells have grown to 70-80% confluency, they were rendered quiescent by incubation for 24hr in DMEM containing 0.5%FCS. During the last 3 hr of the 24 hr incubation , cells were labeled with [methyl-^{3}H] thymidine at 1μCi/ml. (Mean ± S.E.; * $p<0.05$)

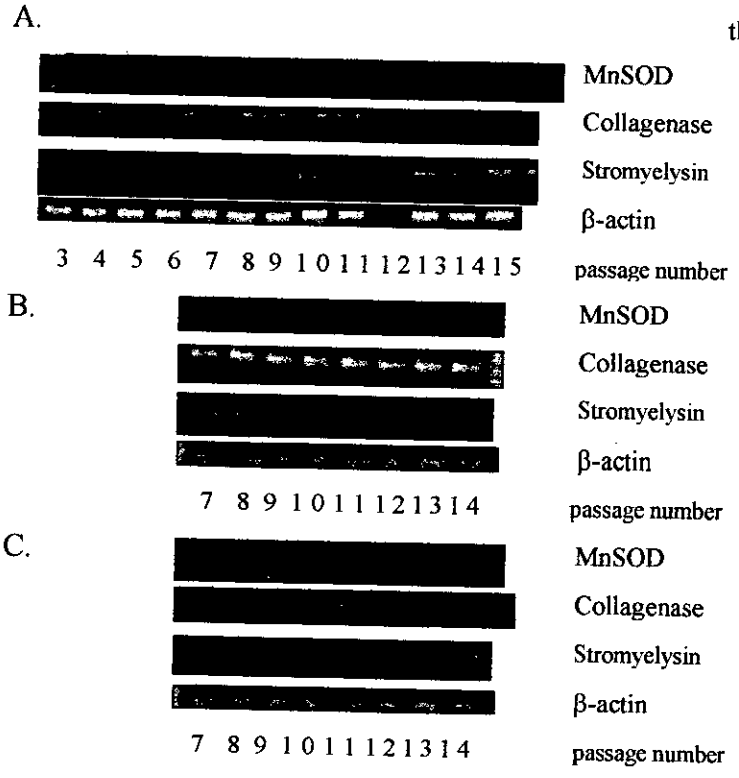

Fig.3 RT-PCR analysis of gene expression in non-transfected GF(A), vector-transfected GF(B), and RII-transfected GF (C). Cells were seeded in 24-well plate at a density of 20000 cells per well. After cell reach confluency, total RNAs from cells were extracted with TRIzol Reagent and reverse transcribed to cDNA. 1/10 of the cDNA obtained was amplified for each mRNA to obtain DNA fragments.