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厦门大学

硕 士 学 位 论 文

重组人胸腺素 a 原对正常小鼠、糖尿病小鼠皮肤伤口愈合过程中 MMP-9/TIMP-1/Nrf2 表达的影响及机制探讨

Influence and underlying mechanism of Prothymosin alpha on skin wound healing of normal and DM mice via MMP-9 /TIMP-1/Nrf2

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摘要

国内外研究表明，胸腺素 a 原（ProTa）具有调节免疫系统、抗氧化、抗感染、抗炎症、抗凋亡、促进细胞分裂、增殖的功能，这为我们开展 ProTa 在正常、糖尿病（diabetes mellitus，DM）鼠皮肤受创后的作用及机理的研究提供了理论依据。

基质金属蛋白酶-9(MMP-9)是一种 IV 型胶原酶，在急性伤口的早期愈合过程中高表达随后下降至正常水平，在整个伤口愈合中发挥着巨大的作用。组织金属蛋白酶抑制剂-1(TIMP-1)是 MMP-9 的特异性抑制剂，在许多细胞因子的分泌和增殖中作用很大。

Nrf2 是调控细胞对抗外来异物和氧化损伤的关键转录因子，可以保护机体免受由高糖高脂引起的氧化损伤。Nrf2 的缺失或激活障碍会引起细胞对应激源的敏感性增高，与化学促癌的发生、炎症修复进程延长、细胞调亡等病变过程密切相关。

ProTa 具有免疫调节和促进细胞增殖的作用，在伤口形成后，各种因子在皮肤愈合过程中是否受 ProTa 调节及其可能机制是什么，以往的研究并没有涉及到，本研究旨在利用体视学、组织学、RT-PCR 和 ELISA 技术阐明 ProTa 对皮肤伤口愈合过程中 MMP-9/TIMP-1、Nrf2 基因和蛋白表达的影响，为研究 ProTa 在促进伤口愈合中的临床应用提供了理论依据，以期为正常、糖尿病创伤的预防和治疗开辟新的途径。本论文分为四个部分。

第一部分为 ProTa 促进正常小鼠皮肤愈合的实验。该部分通过体视学和组织学方法评估 60 、120μg/ml 的 ProTa 处理组与 PBS 对照组伤口愈合效果的情况，取愈合效果最好的 120μg/ml ProTa 处理组设计逆转录聚合酶链反应（reverse transcription polymerase chain reaction，RT-PCR）、酶联免疫吸附（enzyme-linked immunosorbent assay，ELISA）实验，以从 mRNA 水平、蛋白水平检测术后不同时点 MMP-9/TIMP-1、Nrf2、ProTa 在伤口组织中的表达情况。结果表明，120μg/ml ProTa 的愈合效果最好；ProTa(120μg/ml)自身可通过促进血管内皮细胞的增殖来促进皮肤伤口愈合，同时 ProTa 也可能通过抑制 MMP-9、上调 TIMP-1、Nrf2、ProTa 的表达量来加速伤口愈合。

第二部分为 ProTa 促进 DM(由 STZ 诱导)小鼠的皮肤愈合实验：正常小鼠注射 STZ 制作 DM 模型成功后再制作皮肤伤口模型。本部分采用体视学、组织学、Masson 三色染色法、RT- PCR 等方法研究 ProTa 对糖尿病小鼠伤口愈合过程中 MMP-9/TIMP-1、Nrf2、ProTa 的影响。结果表明，ProTa 同样可以促进糖尿病伤口的愈合。

第三部分为 ProTa 促进人脐带静脉内皮细胞增殖的实验，实验结果证明 ProTa 可以促进内皮细胞的增殖。

第四部分为 ProTa 对糖尿病的预防作用研究。本部分实验动物分为雌性组、雄性组，各组再分别随机分为 PBS 对照组、ProTa 处理组。各组先用 ProTa（5μg）腹腔注射 15 天后再用 STZ 诱导糖尿病以破坏胰岛细胞，从生理学水平研究注射 ProTa、STZ 前后小鼠体重、糖耐量的变化。结果发现，ProTa 能够有效的提高雌性组、雄性组小鼠的糖耐量能力，减少体重增加，稳定血糖，同时这种作用不受性别的影响。

我们的研究丰富了 ProTa 在促进细胞增殖、抗凋亡、抗感染、抗氧化等方面的内容，为今后探索临床治疗伤口愈合及糖尿病提供了理论依据。

[关键词] 胸腺素 a 原；伤口愈合；糖尿病

Abstract

Researches both at home and abroad shows that Prothymosin alpha (ProTa) plays a great role in regulating the immune system, antioxidant, anti-apoptosis, resistance to infection and inflammation, that it can promote cell division and proliferation, all of which provides a theoretical basis for us to begin our work which refers to the role and mechanism of ProTa in normal mice and DM mice after skin injured.

Matrix metalloproteinase 9 (MMP-9) is a type IV collagenase involved in early steps of acute wound healing process and found at elevated levels then returns to basal levels in acute wounds. Tissue inhibitor of matrix metalloprotease-1(TIMP-1), a tissue inhibitor of MMPs, has a great effect on the growth and proliferation of many types of cytokines.

Nrf2 is a key transcription factor to protect cells against foreign invaders and oxidative injury, and protect organism against oxidative injury induced by high-sucrose and high-fat. If there is a deletion or activation barriers, cells will very susceptible to stressors, and the processes of inflammatory reparative will be extended, what's more, apoptosis also will happen.

ProTa has the ability to regulate immune system and promote cell proliferation, and MMP-9 has an intimate relationship with wound healing and scarring, Nrf2 plays a great role in anti-oxidizing, whether ProTa has an effect on expression of MMP-9, TIMP-1, Nrf2 and what's the underlying mechanism when these factors exist simultaneously? However, the former researches have not referred so far. In our study, we used technology such as stereology, histology, RT-PCR and ELISA to illuminate the effect of ProTa on the expression and secretion of MMP-9/TIMP-1, Nrf2 in a murine wound healing model, which will provide a strong theoretical basis for clinical application.There is four parts in our work.

The first part refers to ProTa promote wound healing process in normal mice. In

this part, we use technologies such as stereology,histology to evaluate the healing effect of 60 ,120μg/ml ProTa treated groups and PBS control group.120μg/ml ProTa treated group, the best healing effect group ,was designed to examine the expression and secretion of MMP- 9/TI MP- 1 ,Nrf2,ProTa at different time points during wound healing ,using technology such as RT-PCR,ELISA. Results show that ProTa (120μg/ml) can promote wound healing by itself through promoting the formation of proliferation of vascular endothelial cell. Meanwhile, ProTa promotes wound healing maybe through down-regulating the expression of MMP-9 and up-regulating the expression of TIMP-1, Nrf2 and ProTa.

The second part is ProTa promote wound healing process in DM mice. Normal mice were injected with STZ to produced diabetes mellitus model, on the dorsum of which were made a circular (12mm) full-thickness excisional wound. We used technologies such as stereology, histology, Masson's Trichrome Stain, RT-PCR to examine the expression and secretion of MMP- 9/TIMP-1, Nrf2, and ProTa at different time points during wound healing. Results show that ProTa also can promote wound healing of DM mice.

The third part proved that ProTa can promote the growth and proliferation of vascular endothelial cells in vitro.

The fourth part mainly focus on the function of ProTa to protect organism against DM. Animals in this part was divided into female group and male group, which were randomly divided into PBS control group and ProTa treated group respectively. Both PBS control group and ProTa treated group were injected intraperitoneally with ProTa (5μg) 15 days, then STZ to induce DM model and damage to islet cells. Body weight and glucose intolerant ability were examined to evaluate the effect of ProTa to organism. We found that ProTa could enhance effectively glucose intolerant ability of both female and male mice, and that prevent over weight, regulate the glucose. What's more, this effect did do work to female and male mice.

Our studies will rich the functions of ProTa, such as promote cell division and proliferation, anti-apoptosis, resistance to infection and inflammation, which will