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硕士学位论文

Pygo2通过Wnt信号通路影响小鼠脂肪发育的研究

Pygo2 affects the adipogenesis of mice by Wnt signaling pathway

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摘 要

越来越多的实验研究表明，Wnt信号通路在脂肪细胞的生长与分化过程中具有重要的调节作用。脂肪组织作为人类重要的能量储存器官，其发生与发展与人类的健康息息相关。然而，随着人类生活水平的的提高，能量摄入的增加和运动量的减少及其环境等因素的影响，导致患肥胖症人群在全世界范围内普遍增多。而另一方面，脂肪组织不仅仅只作为储存能量的重要器官，并且够分泌多种激素类物质，其脂肪细胞的分化及其调控发生异常通常都会伴随着各种各样疾病的产生，如高血脂症、糖尿病、脂肪肝以及乳腺癌等。

目前，已有大量研究表明Wnt信号通路在参与脂肪组织生长发育的调控过程中扮演着重要的角色，然而Pygopus2作为Wnt信号通路中的一个重要转录因子，其调控脂肪形成的研究目前还处于空白。

在本课题中，我们发现Pygo2敲除的小鼠胚胎成纤维细胞（mouse embryonic fibroblasts（MEFs））在体外条件下培养能发生自分化而形成成熟脂肪细胞。根据这一现象，使我们对Pygo2是否参与调控脂肪形成这一问题产生了兴趣。由于Pygo2全身敲除小鼠胚胎致死，我们通过利用Pygo2脂肪组织特异性敲除小鼠AP2-cre&Pygo2flox/flox，对其在脂肪形成发育过程中进行观察研究。通过对小鼠体重测量，组化切片染色等一系列检测，我们发现，Pygo2敲除的小鼠脂肪组织表现出了组织肥大的特点。而对小鼠脂肪组织RNA提取，并通过实时定量PCR检测，显示Pygo2敲除小鼠脂肪分化相关标准蛋白含量有较明显的上升。而在细胞水平上，我们发现，Pygo2在3T3-L1前脂肪细胞正常分化成熟过程中的表达含量逐渐减少。此外，通过RNA干扰技术敲低3T3-L1细胞中Pygo2的表达能促进其分化，而过表达Pygo2能抑制3T3-L1前脂肪细胞的分化。最后，我们通过荧光素酶报告基因实验，验证了Pygo2通过Wnt信号通路抑制了脂肪细胞的分化形成。

关键词：肥胖；Wnt信号通路；Pygopus2

Abstract

More and more studies showed thatWnt signaling pathway plays a significant role in regulation of cell proliferation and differentiation. As an energy storage organ, fat tissue's development and progression is bound up with human health. However, with the improvement of human living standards, they have more opportunities to obtain energy but lower chance to take them into sport, Hence, the number of obese people are on the rise worldwide. Besides, fat tissue is not only as an organ for energy storage,but also plays an important role in secreting many kinds of hormones or hormone-like peptides.So if there are something abnormal occur in differentiation and regulation of adipocyte that will company with all kinds of disease in human body, such as hyperlipidemia, diabetes, fattyliver,and breastcancer.

At present, a large number of studies have shown that Wnt signaling pathway in the growth and development of adipose tissue plays an important role in its regulation, but Pygopus2 as an important transcription factor in the Wnt signaling pathway, its regulation of fat formation is still in the blank.

In this study, we found that, in vitro, Pygo2 deficient mouse embryonic fibroblasts (MEFs)occurred self-differentiation after contact suppression and become mature adipocyte. According to this phenomenon, we are interested in whether Pygo2 is involved in regulating fat formation.Since Pygo2 systemic elimination of mouse is embryos lethal, we knocked out Pygo2 in adipose tissue conditionallyby using aP2-cre, Pygo2flox / flox mouse. By measuring the weight, histological sections ,HE staining, and other method, we found hypertrophy occur in Pygo2 deficient mice fat tissue. Through extracting mice fat tissue RNA and measuring its fat-relative marker protein by real-time PCR , we found ,compare to wildtype mice, the content of this protein is increased significantly in Pygo2 deficient mice fat tissue.

At the cellular level, we found that knockdown Pygo2, by using RNA interference, can promote 3T3-L1 pre-adipocytes,and inhibitits differentiation by overexpression of Pygo2.

Keywords: Adipocity; Wnt signaling pathways; Pygopus2;.

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