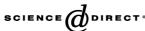


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Transglutaminase 2 expression in the salivary myoepithelial cells of mouse embryo

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KEYWORDS

Transglutaminase 2; Myoepithelial cell; Submandibular gland; Mouse embryo

Summary Earlier a strong transient expression of transglutaminase 2 (TGase 2) localized at the anchoring sites of muscle bundles in human embryo was observed (Lee SK, Chi JG, Park SC, Chung SI. Transient expression of transglutaminase C during prenatal development of human muscles. J Histochem Cytochem 2000;48:1565-1574). In this study, we report a similar transient expression of the TGase 2 in the salivary myoepithelial cells of mouse embryo by immunohistochemistry, RNA in situ hybridisation, and RT-PCR. From 35 submandibular glands of mouse embryos and postnatal mice, a consistent expression of TGase 2 in the myoepithelial cells via a stage-specific manner was identified by mono-clonal antibody to TGase 2 immunostaining. A similar expression pattern of TGase 2 in the myoepithelial cells was also observed by RNA in situ hybridisation analysis. The expression of TGase 2 in the salivary epithelium and mesenchyme during the prenatal 14.5-15.5 days was found minimally diffusely spread and became intensely focalised in the myoepithelial cells of salivary acini and ducts during the prenatal 16.5-18.5 days but thereafter gradually decreased until postnatal 7 days and remained weak in postnatal 3 weeks. Such transient rise and fall expressions of TGase 2 were also found with the sequential amount of RT-PCR products during the same period. The α -smooth muscle actin (α -SMA) as a positive control in the myoepithelial cells of mouse submandibular glands was consistently expressed during the prenatal and postnatal period. These results of transient expression of TGase 2 in the myoepithelial cells coincided with the formation of the dendritic basket structure in the periphery of acini and ducts, suggest a possible catalytic role of transglutaminase in a newly formed cellular matrixes during the cytodifferentiating stage of mouse prenatal and neonatal submandibular glands. © 2004 Elsevier Ltd. All rights reserved.

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Introduction

Transglutaminases (TGases) are a widely distributed group of enzymes that catalyse the post-translational modification of proteins by the formation of isopeptide bonds. This occurs either through protein cross-linking via epsilon-(gamma-glutamyl)lysine bonds or through incorporation of primary amines at selected peptide-bound glutamine residues. The cross-linked products, often of high molecular mass, are highly resistant to physical stress and proteolytic degradation, and their accumulation is found in a number of tissues and processes where such properties are important, including skin, hair, blood clotting and wound healing. However, deregulation of enzyme activity generally associated with major disruptions in cellular homeostatic mechanisms has resulted in a number of human diseases, including chronic neurodegeneration, neoplastic diseases, autoimmune diseases, diseases involving progressive tissue fibrosis and diseases related to the epidermis of the skin.²

TGase 2 (tissue TGase, TGase C) is known as an ubiquitous cross-linking enzyme involving different cellular progresses, i.e., proliferation, differentiation, and apoptosis, and it is utilized even in the lower animals, plants, and microorganism. 3-6 However, the finding that the TGase 2 knockout mice did not display any abnormal growth, rather they survive well in postnatal period^{7,8} leaves many perplexing questions. The normal development of TGase 2 knockout mouse might be that the compensatory replacement of the cross-linking enzymes by other TGase isozymes⁸ as was observed with other enzyme knockout animals having isozymes. It was also reported that the over-expression of TGase 2 frequently produced apoptotic change both in vivo and in vitro experiments^{7,9} and induced cardiac hypertrophy. 10 Furthermore, the TGase 2 would be clearly relevant to the Celiac diseases and other autoimmune diseases, including osteoarthritis and renal scarring (Table 1). $^{11-15}$

In the previous study, we observed the stagespatial expression of TGase 2 in the early developmental stage of human salivary gland, especially localized in the ductal luminal cells when the salivary gland underwent branching and tubulisation, suggesting a possible role of TGase 2 as a major cross-linking enzyme, involved in the stabilization of the luminal surface of the salivary gland. 16 Recent findings of the strong temporal TGase 2 expression in the myoblasts of skeletal, smooth, and cardiac muscles of human embryos and foetuses¹ further added support for our hypothesis of TGase 2 as an important enzyme participating in the stability of formed extracellular sheeth/matrixes during the differentiation of myepithelial cells in the embryonic organogenesis. 17-19 In this study we examined the submandibular glands of mouse embryos, which showed relatively shorter developmental period than human foetus, and found that the TGase 2 was transiently expressed in the myoepithelial cells of submandibular gland during the early developmental stage.

Materials and methods

Immunohistochemistry

The pregnant mouse which had a vaginal plug in the morning following mating was considered embryonic 0.5 day (E0.5). Submandibular glands were removed from 14.5 (n = 2), 16.5 (n = 3), 17.5 (n = 3), 18.5 (n = 3) days old mouse embryos, and 1 (n = 5), 3 (n = 5), 5 (n = 5), 7 (n = 4) days and 3

Table 1	Immunohistochemical localisation of TGase 2 and α -SMA in the mouse prenatal and postnatal submandibular
glands.	

	TGase 2			α-SMA	
	Number	Myoepithelial cells	Acinar/ductal cells	Myoepithelial cells	Acinar/ductal cells
Embryonal					
14.5 day (E14.5)	2	+	_	++	_
16.5 day (E16.5)	3	++	_	++	_
17.5 day (E17.5)	3	+++	±	+++	_
18.5 day (E18.5)	3	++	±	++	_
Postnatal					
1 day	5	++	±	+++	_
3 day	5	++	±	+++	_
5 day	5	+	±	+++	_
7 day	4	+	±	+++	_
3 week	5	+	±	+++	_
Total	35				

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