

Expression of Cx genes in liver and stomach of different embryonic stages *

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Abstract

AIM To explore the relationship between the rules of Cx gene expression and cellular differentiation in organs of different embryonic stages.

METHODS A series of Cx gene serving as molecular probes and the Northern blot hybridization were employed to study the Cx gene expression.

RESULTS Cx31, Cx31.1, Cx46 did not express while other Cx genes expressed in the embryonic liver and stomach. The Cx gene expression in the liver and stomach showed different state at different embryonic stages. The Cx gene expression had organic diversity. The expression of Cx26 gene was overlapping in the above organs. Cx43 did not express in the human liver after birth, but it expressed in the embryonic stage.

CONCLUSION The expression state of Cx genes is concordant with cellular differentiation. It might be a key candidate gene to regulate some differentiations events associated with cellular differentiation, proliferation, and morphogenesis in the early embryo.

INTRODUCTION

Cellular connexin genes are a multigene family consisting of more than 10 members^[1-4] which encode the gap junctional channel assembled protein, connexin (Cx). The latter is the key composite of gap junctional intercellular communication (GJIC). The expression of Cx genes determines the formation, pattern, amount and the degradation of GJIC channel. The specific connexin proteins transcribed and translated by different members of Cx gene family contribute to the diversity of gap junctional channel within different tissue cells or the same type of cells but in different functional states, leading to the differences in patterns and structures of gap junction. The members of connexin gene are related to the type and size of communication channel and communication ways of gap junction^[5]. Gap junction is present in the early stage of embryonic development. The morphological study has verified that gap junction is detected within morula and blastocyst, and dense GJICs appear in cells of embryonic ectoderm at 20 h - 30 h of developing chicks^[5]. In order to investigate the relationship between the expression law of Cx genes and cellular differentiation in the liver and stomach during embryonic development, the Cx gene expression state was studied using Cx26, Cx31, Cx31.1, Cx32, Cx37, Cx40, Cx43 and Cx46 as molecular probes and the Northern blot hybridization.

MATERIALS AND METHODS

Materials

The liver and stomach derived from the fetals of mothers receiving natural abortion with conceptional age of 5 weeks, 2, 3, 4 and 5 months. The tissues or organs were stored in fluid nitrogen.

Methods

Preparation of plasmids containing Cx cDNA A small amount of bacteria containing 8 kinds of pCx plasmids and inner control pGAPDH plasmid was recovered. An inoculum of bacteria was streaked on an agar plate and incubated overnight at 37°C. The next day single bacterial colonies were picked for amplification; plasmids were extracted with alkaline lysis procedure and digested and identified with appropriate restrictive endonuclease. The termini were then isolated by electrophoresis in 10g/L agarose gel and retrieved and purified with Glass Max kit (Table 1).

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Table 1 A list of plasmids containing Cx cDNA

Plasmids	Vectors	Restriction enzymes	Inserts (kb)	Species
pCx26	pSG5	<i>Bam</i> H I	0.68	human
pCx31.1	SP63T	<i>Bgl</i> II	0.85	mouse
pCx32	pGEM3	<i>Eco</i> R I	1.5	rat
pCx33	SP64T	<i>Bgl</i> II	0.9	rat
pCx37	SP64T	<i>Hind</i> III/ <i>Xba</i> I	1.25	mouse
pCx40	pGEM4z	<i>Eco</i> R I/ <i>Xba</i> I	1.1	mouse
pCx43	pSG5	<i>Bam</i> H I	1.11	human
pCx46	BSK+	<i>Eco</i> R I	1.6	human
pGAPDH		<i>Eco</i> R I/ <i>Hind</i> III	0.6	human

Table 2 Expression of Cx genes in different organs at different fetal ages

Connexin genes	2 month		3 month		4 month		5 month	
	Liver	Stomach	Liver	Stomach	Liver	Stomach	Liver	Stomach
Cx26	++	+	+++	+	++	+	+	+
Cx31	-	-	-	-	-	-	-	-
Cx31.1	-	-	-	-	-	-	-	-
Cx32	+++	-	+++	+	+++	++	+++	+++
Cx37	-	++	-	+++	-	-	-	++
Cx40	-	+	-	++	-	-	-	+++
Cx43	++	-	+++	-	++	++	+	++
Cx46	-	-	-	-	-	-	-	-

+++ : high, ++ : moderate, + : low, - : no or weak

Preparation and purification of total RNA

Total RNA was extracted by acid guanidinium-phenol chloroform procedure from the liver and stomach in different stages of embryo respectively.

Northern blot

Total RNA (20μg in each well) was separated by electrophoresis through formal dehyde denaturing gels and transferred onto nylon membranes by capillary transfer for 24 h in 20 × SSC. The RNA was cross-linked to the membranes by exposure to UV light.

Northern hybridization between Cx gene probes and RNA

Connexin-specific cDNA probes were random-prime labeled with [α -³²P] dCTP. The membranes were prehybridized at 42°C for 4h in Northern hybridization solution (500 g/L-formamide, 5 × SSPE, 5 × Denhardt's reagent, 1 g/L-SDS solution and 100 mg/L salmon sperm DNA) and then hybridized with the labeled probe for 16 h - 24 h in the same solution.

Autoradiograph (ARG)

The membranes were washed twice at 37°C in

2×SSC/ (1 g/L)-SDS for 40 min and three times at 65°C in 0.1 × SSC/ (1 g/L) SDS for 1 h. Exposure of X-ray film to the blots was conducted at -70°C with intensifying screens. To remove probes from the membranes, the membranes were immersed in 1 g/L- SDS solution in rotating platform from boiling to RT. The process was repeated. Then the membranes were rehybridized with GAPDH probe.

RESULTS

Cx31, Cx31.1 and Cx46 did not express while other Cx genes expressed in the liver and stomach of embryonic stage. The expression of Cx genes showed different states in the liver and stomach at different embryonic stages. The expression of Cx genes was of organic diversity, e. g., the expression of Cx32 in the liver and Cx37 in the stomach. The Cx gene expression was overlapping in the above organs, and Cx26 presented different states of expression. Cx32 gene had high expression from the 5th week to the 5th month in the liver and the 4th to 5th month in the stomach. Cx43 did not express in human liver after birth, but it expressed in the fetal stage. Cx37 gene in the stomach of fetals highly expressed from the 3rd month to the 5th month (Table 2).

DISCUSSION

Cx genes showed different expression states, e.g., Cx26, Cx32, Cx43 in the liver, and Cx26, Cx32, Cx37 and Cx43 in the stomach. The diversity of expression indicated that the various gap junctional channels between different cells or the same cells in different functional state was due to the expression of different members of Cx gene family^[6].

Cx26 gene in the fetal liver had weak expression in the 5th week, and high expression in the 3rd month, which lowered gradually during the 4th to 5th month. The expression and nonexpression of Cx26 gene are believed to be relevant to morphogenesis of hepatic lobule, central vein and portal area. There was no structure of hepatic lobule, central vein and portal area in the liver in the 5th week of embryo. The structures formed gradually during the 2nd month, and hepatic lobule was recognized in the 3rd month. Cx26 was in its high expression state during this stage. The structure of portal area became clear in the 4th month. Up to this stage, the Cx26 expression gradually reduced when the basal morphological structure of liver was established. The Cx26 gene expression was overlapping in the stomach, which might be related to the morphogenesis of stomach. It is suggested that the Cx26 expression plays an important role in the morphogenesis and structural building of the liver and stomach during embryogenesis.

The expression state of Cx genes is conformed to the cellular differentiation. It may be related to its own differentiation and proliferation of liver cells. This is compatible with Zeng's report in which the cells of primeval region will acquire further differentiative capacity, which is closely related to a high level of expression of Cx genes among the same cells^[7]. Cx32 gene expression in the stomach is related to development of gastric gland, it was low during gastric gland bud stage in the 3rd month, and increased gradually during the developing stage of primordial gastric gland in the 4th month, and reached a peak in the 5th month when the gastric gland grew completely.

Eghbali^[8] transfected cDNA of whole length Cx32 gene into the hepatocellular cancer lacking Cx32, and found mRNA expression level, and gap junction were increased markedly, and ion coupling and metabolite coupling reappeared in the tests of dye transfer and electric current of intercellular communication, meanwhile the growth rate of car-

cinoma cells transfected with Cx32 decreased rapidly, and cellular structure of neoplastic cells differentiated towards normal cells. It is indicated that the expression of Cx32 gene plays a significant role in the cellular differentiation.

Cx43 does not express in the human liver after birth^[9], but it expresses in the fetal stage. It may be related to hematopoietic function of the liver during the fetal stage. Hematopoietic stem cells migrate to liver from yolk sac at early embryonic stage; at this time, Cx43 expression is low and turns high when the weight of hematopoietic tissues amounts to 30%-40% of the liver by the 3rd to 4th month of embryonic development. With the formation of the spleen, thymus and bone marrow, most of the hematopoietic stem cells in the liver migrate to the above organs, and the Cx43 gene expression lowers distinctly in the 5th month of the fetal liver, indicating the lowered hematopoietic function of the liver. It may be related to the smooth muscle development in stomach^[4].

Wang SQ^[10] held that gap junction appeared in the early embryonic stage made it possible the intercellular transfer of substances which regulated cellular differentiation, morphological formation and growth control. For example, in archigastrula and neurula, gap junctions made notochord-induced ectoderm develop into neural tubes. During embryonic development, the expression state of Cx genes is basically concordant with the formation and degradation of gap junctional communication channel. It is suggested that Connexin gene may be a key candidate gene to regulate some differentiations events associated with cellular differentiation, proliferation and morphogenesis and organ development.

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