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• BASIC RESEARCH •

Localization of TRAIL/TRAILR in fetal pancreas

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Abstract

AIM: To observe the localization of TRAIL/TRAIR (DR4, DR5, DcR1, DcR2) in the fetal pancreas.

METHODS: Fetal pancreas of 32 weeks of pregnancy were obtained from induced abortions, embedded in paraffin, and 4-μm sections were prepared. The localization of TRAIL/TRAILR in fetal pancreas was investigated by fluorescence immunohistochemical method combined with laser scanning confocal microscopy.

RESULTS: TRAIL immunoreactive cells were mainly located on the periphery of the pancreas islets. There were a few DcR1 and DcR2 positive cells whereas there were no immunoreactive cells of DR4 and DR5 in the pancreas islets. In the acini and the ducts of the exocrine pancreas there were no TRAIL/TRAILR immunoreactive cells.

CONCLUSION: This study not only describes the distribution of TRAIL/TRAILR in the fetal pancreas, but also provides a morphological basis for deducing the function of TRAIL/TRAILR in pancreas, suggesting that in normal pancreatic islets, the pancreatic cells are resistant towards apoptosis too.

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INTRODUCTION

Being an important endocrinology organ, the pancreas plays an important role in many physical and pathological processes[1-11]. The pancreatic islets could secrete not only the classical endocrinology substances such as insulin, glucagons, somatostatin and so on, but also could secrete some neuropeptides and cytokines[12,13].

Tumor necrosis factor related apoptosis inducing ligand (TRAIL)/TRAILR is a group of molecules belong to TNFSF/TNFRSF identified from 1995 and displays very important biological function[14-20]. Despite the widespread interest in TRAIL and its receptors to date, studies on TRAIL/TRAILR are mostly stayed on mRNA level just for the lack of monoclonal antibodies. Fortunately, a series of antibodies against TRAIL/TRAILR system have been prepared and identified successfully by our department and Screaton group recently and these provided a useful way to detect the expression of TRAIL/TRAILR in many organs and tissues[21]. In this experiment, we detected the localization of TRAIL/TRAILR in fetal pancreas.

MATERIALS AND METHODS

Reagents

The mouse anti-human DR4, DR5, DcR1, and DcR2 antibodies and the rabbit anti-human TRAIL antibody were prepared in our department. Biotin conjugated horse anti-rabbit IgG, FITC conjugated goat anti-mouse IgG and Texas red-conjugated streptavidin were purchased from Sigma.

Preparation of tissue sections

Five fetal pancreas of 32-week pregnancy were surgically obtained. They were washed with physiological saline at 4 ℃ and a 6-mm^3 piece was taken from each before fixed in Bouin' s solution overnight. Each piece was embedded in paraffin and 4-μm sections were prepared.

Immunohistochemistry

Four-micrometer sections from fetal pancreas of 32-week pregnancy were employed in the fluorescent immunohistochemical analysis of TRAIL/TRAILR. Several dilutions of the antibody were tested to find the optimal staining concentration before the entire series was processed. The staining procedure was carried out as previous reports, without protease treatment[22]. Briefly, the steps included: (1) the sections were deparaffinized in xylene, hydrated in ethanol, and washed in 0.01 mol/L PBS, then pretreated with 30 mL/L normal goat serum for 40 min and rinsed in 0.1 mol/L PBS; (2) incubation at 4 ℃ for 24 h in the primary antibodies, mouse anti-human DR4, DR5, DcR1 and DcR2 antibody (1:200 dilution, final concentration of 25 mg/L) was performed respectively, and then in rabbit anti-human TRAIL antibody (1:300 dilution in 10 mL/L BSA-PBS); (3) the secondary antibody, biotin-labeled horse anti-rabbit IgG (1:200 dilution), was incubated at room temperature for 1 h; (4) simultaneous incubation with 1:200 FITC conjugated goat anti-mouse IgG and Texas red-conjugated streptavidin (1:1 000 dilution) for 30 min. The sections were washed three times for 10 min each after incubation from steps 2 to 4, respectively, and were finally mounted in 50 g/L glycerin. The sections were examined with Bio-Rad 1024 LSCM. The specimens were excited with a laser beam at wavelengths of 568 nm (Texas Red) and 488 nm (FITC) and the emission light was focused through a pinhole aperture. The full field of view was scanned in square image formats of 512x512 pixels.

Controls

Primary antibodies were substituted by irrelevant antibodies and normal rabbit or goat serum as specific antibody control. PBS was substituted for primary antibody as negative control. Primary antibody was omitted as blank control.

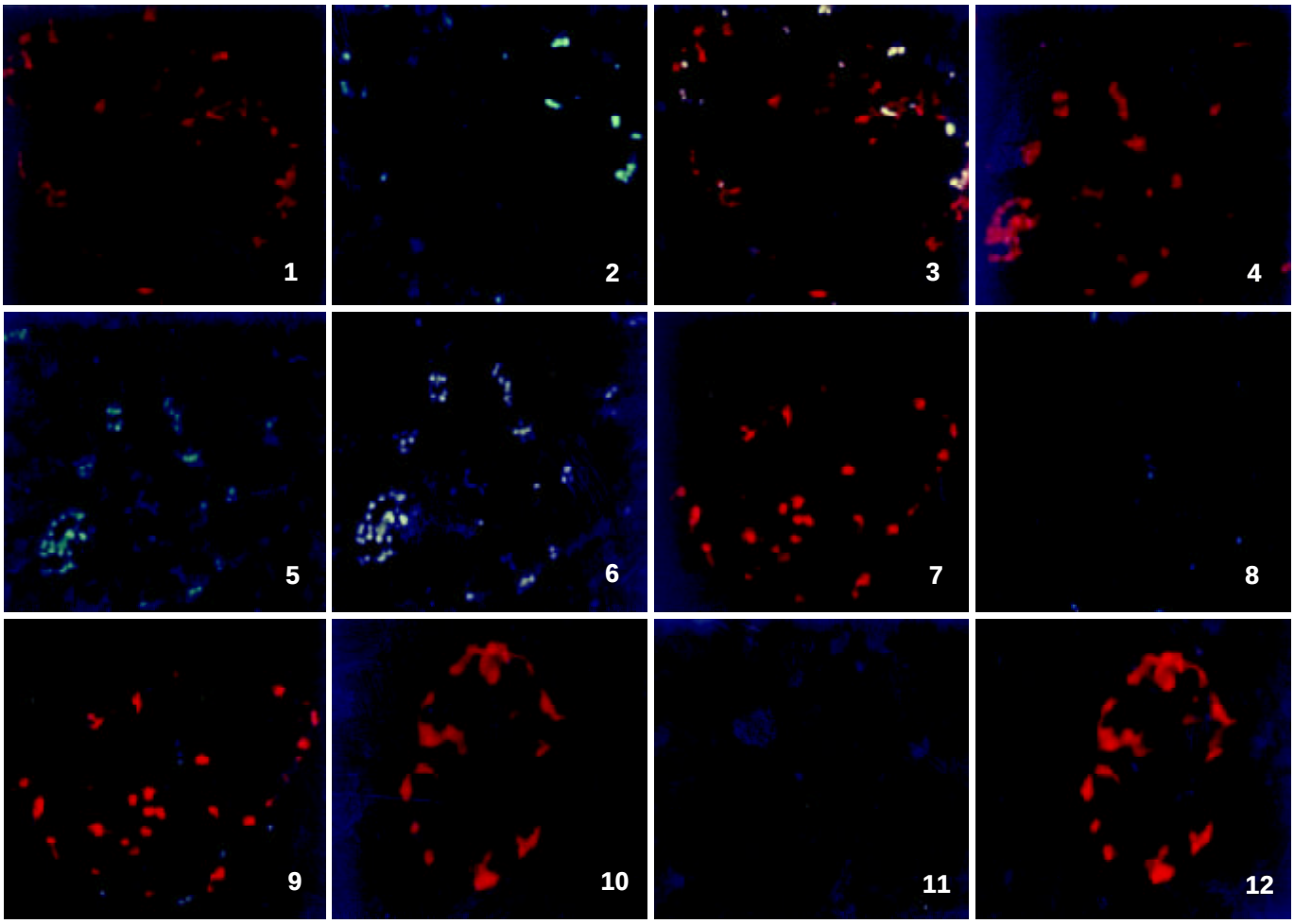

Figure 1, 4, 7, 10 Distribution of TRAIL in fetal pancreas. The positive reactivities are mainly distributed in membrane (red). TR-labelled ×400.

Figure 2, 5, 8, 11 Localization of DcR1, DcR2, DR4 and DR5 respectively in fetal pancreas. There are a few DcR1 (Figure 2) and DcR2 (Figure 5) positive cells and the positive substances locate in membrane mainly (green). There is no DR4 (Figure 8) and DR5 (Figure11) positive cells distributed in fetal pancreas. FITC-labelled ×400.

Figure 3, 6, 9, 12 Coexpression of TRAIL / TRAILR in fetal pancreas. Some TRAIL positive cells coexpress with DcR1 or DcR2, whereas there is no coexpression of TRAIL and DR4, TRAIL and DR5 in fetal pancreas. The positive cells distribute in disperse (yellow). TR-labelled TRAIL, FITC-labelled TRAILR (DcR1, DcR2, DR4 and DR5) ×400.

RESULTS

Localization of TRAIL in fetal pancreas

Fluorescent immunohistochemistry showed that TRAIL positive cells located on the periphery of pancreatic islets mainly. In the acini and the ducts of the exocrine pancreas there were no TRAIL immunoreactive cells. The positive cells distributed in disperse or patch pattern. The positive reactivity was mainly distributed in membrane and cytoplasm, while nuclei remained immunonegative (Figures 1,4,7 and 10).

Localization of TRAILR in fetal pancreas

Figures 2, 5, 8 and 11 showed the localization of DcR1, DcR2, DR4 and DR5 respectively in fetal pancreas. There were a few DcR1 (Figure 2) and DcR2 (Figure 5) positive cells and the positive reactivity located in membrane mainly (green). There was no DR4 (Figure 8) and DR5 (Figure 11) positive cells distributed in fetal pancreas.

Coexpression of TRAIL and TRAILR in fetal pancreas

Some TRAIL positive cells coexpressed with DcR1 or DcR2, whereas there was no coexpression of TRAIL and DR4, TRAIL and DR5 in fetal pancreas. All DcR1 and DcR2 immunoreactive cells showed TRAIL immunoreactivity (Figures 3, 6, 9 and 12).

DISCUSSION

Members of the TNF and TNF superfamilies of proteins are involved in the regulation of many important biological processes, including development, organogenesis, and innate and adaptive immunity[23]. The TNF-related apoptosis-inducing ligand (TRAIL, also known as Apo-2L) is a newly identified TNF superfamily member with high homology to FasL. In the TRAIL/TRAILR system, control over apoptosis relies on differential display of receptors (TRAILR). These include DR4 (TRAIL-R1) and DR5 (TRICK2/ TRAIL-R2), which transduce apoptotic signals, as well as DcR1 (TRID/LIT/ TRAIL-R3) and DcR2 (TRUNDD/TRAIL-R4), which lack functional death domains and act as decoys. Osteoprotegerin (OPG), a soluble decoy receptor for OPGL/RANKL/TRANCE, which is involved in osteoclast function, has also been reported to bind to TRAIL[24-36].

There were some reports on the distribution of TRAIL/ TRAILR in human pancreas and pancreatic cancer. Liao *et al* found that TRAIL-R3 mRNA and protein expression were generally weak in pancreatic cancers and normal pancreatic tissues. In contrast, TRAIL-R4 mRNA and protein were expressed at moderate to high levels in human pancreatic cancer tissues, but demonstrated weak to negative expression in the normal pancreas by Northern blotting, Western blotting and

immunohistochemistry[37]. Satoh *et al* detected the expression of TRAIL and its receptors other than osteoprotegerin in normal pancreatic tissues using RT-PCR[38]. In our experiment, we found for the first time that in fetal pancreatic islets, there was strong distribution of TRAIL protein. The TRAIL immunoreactive substances mainly located on the membrane and cytoplasm with negative nuclei. Some TRAIL immunoreactive cells also showed DcR1 or DcR2 positive immunstaining, whereas there were no expression of DR4 and DR5 in fetal pancreas. Our results show some consistence with what Liao et al and Satoh et al have found.

We all know that pancreatic cancer is one of the most aggressive cancers, partly due to the general resistance of pancreatic cancer cells towards apoptosis. Ibrahim *et al* demonstrated that pancreatic carcinoma cells evaded the immune system in two mechanisms. One is the expression of nonfunctional receptors, decoy receptors, and molecules that block cell death, such as bcl2 and bcl-Xl. The other is the expression of apoptosis-inducing ligands, such as TRAIL, that could induce cell death of immune cells[39]. In our experiment, we found that in fetal pancreatic islets, some cells expressed TRAIL. At the same time, DcR1 and DcR2 also colocated with TRAIL in some cells, but there was no expression of DR4 and DR5. Our findings suggest that in fetal pancreatic islets, the pancreatic cells are resistant towards apoptosis too. The characteristics of the fetal pancreas are consistent with those of pancreatic cancer.

It is well known that there are some kinds of cell types in human pancreatic islets such as A cells, B cells, D cells and PP cells. Although we found that TRAIL was located in some endocrine cells, we could not conclude which kind of cell types it existed in. This still needs to be clarified further.

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