

## The Experimental Study for Heat Shock Protein 70 and Acute Rejection After Bowel Transplantation in Rat

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**Abstract Objective:** To investigate the relationship between heat shock protein 70 and acute rejection after bowel transplantation in rat. **Methods:** SD to Wistar rat and Wistar to Wistar rat juxtaposition type small bowel transplantation were established in our experiment. The grafts were got on the posttransplantation day 1, 4, 7 and were used to detect the expression HSP70 by immunohistochemistry. The results were compared with the acute immuno-rejection levels. **Results:** High expression of HSP70 in SD to Wistar rat group. The expression of HSP70 has close relation to acute immuno-rejection levels. **Conclusions:** HSP70 plays an important role in acute rejection of bowel transplantation in rat.

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**Key Words:** Heat shock protein 70; Small bowel transplantation; Rejection

Heat shock protein 70 (HSP70) exist widely in prokaryotes and eukaryotes, there is a natural expression of rat small bowel, a variety of stimulating factors can obviously induce heat shock protein occurrence and heat shock response expression, has important significance in stress response in defense mechanism. Recent studies show that HSP70 in antigen presenting plays an important role in assisting the antigen presenting related molecular assembly, closely related with immune response<sup>[1]</sup>. This study used immunohistochemical S-P method to detect the expression HSP70 of bowel transplantation in rat, to explore the expression HSP70 and its reasons in acute rejection after bowel transplantation in rat.

### 1 Materials and methods

#### 1.1 The experimental animal and grouping

We prepared inbreeding group adult male SD rats, Wistar rats (by the experimental animal research center of Sichuan Academy of Medical Sciences), weighting 250 ~ 280g. Establish rat juxtaposition type small bowel transplantation of 20 cases in each. Experimental group: SD to Wistar rats allotransplantation group. Control group: Wistar rats in isotransplant group.

#### 1.2 Establishment of rats model of small bowel transplantation

The donor operation: abdominal incision like "+", separate small bowel mesentery from the colon, ligate the arteriae colica media, separate pancreas from the superior mesenteric vein and portal vein. Dissociate abdominal aorta, ligate the proximal by silk thread, use 7 size indwelling needle infusion of heparin Ringer's solution in the distal, mutilate portal vein in the hepatic portal, mutilate all the small bowel

at the flexura duodenojejunalis and terminal ileum. Lavage enteric cavity from the near end down by 4°C Ringer's solution, store the donor bowel in 4°C Ringer's solution. Recipient operation: via the ventral midline incision to the abdomen, dissociate inferior vena cava and the abdominal aorta, donor and recipient abdominal aorta distal were side anastomosed below the renal artery, the portal vein and the recipient inferior vena cava were side anastomosed.

#### 1.3 The sampling, fixation, preservation

On the posttransplantation day 1, 4, 7 successively cutted fistulization of bowel respectively from ileostomy to the proximal, 2 segments each time, each segment of 2cm, and 10% formalin fixed retained to immunohistochemistry and pathological examination.

#### 1.4 Main reagents

Rat anti rabbit HSP70 monoclonal antibody (BA0928, purchased from boster Biological Engineering Co., Ltd.). Ultra Sensitive™ S-P kit, purchased from Fuzhou Maixin biological Technology Development Company.

#### 1.5 Test method

We used immunohistochemistry to detect the expression of HSP7. The results were compared with the acute immuno-rejection levels by routine pathological HE staining.

#### 1.6 Immunohistochemical experiment steps

Sections were dewaxed routinely, xylene and graded alcohol used to dehydrate. After heat repaired via the microwave oven, cleaned by PBS, and use 10% normal goat serum to reduce nonspecific antibody binding. Dropwise first antibody and save in the 4°C wet box for the night. After cleaned by the

PBS solution, adding biotinylated two antibody and horseradish peroxidase labeled avidin. DAB coloration, hematoxylin staining, 1% hydrochloric acid alcohol color separation, gradient alcohol dewatering, and xylene transparent, fixed, microscopic examination.

### 1.7 The judgment standard

HSP70 expressed both in cytoplasm and nucleus, early after the operation in the cytoplasm, later period mainly in nucleus. The cells were clearly brown as positive, no brown cells or light brown background similar as negative. According to the proportion of positive cells divided as follows: +: meaning the number of positive cells<25%,++: meaning the number of positive cells25%~50%,+++ meaning the number of positive cells>50%. References formulate pathological diagnosis standard of small bowel immune rejection grading [2]: Mild rejection: the number of intestinal mucosal villi reduced, mild edema, villi become shorter, with a small amount of inflammatory cell infiltration in the stroma. Moderate rejection: the intestinal villus epithelial scarce, began to fall, the number of goblet cells and Paneth cells decreased, inflammatory infiltrate aggravated mainly by the lymph and monocytes, appearing slightly necrosis.: Severe rejection: the disappearance of the intestinal villus structure, mucosal epithelial fall off completely, the intestinal wall became thin and necrosis, goblet cells and Paneth cells disappeared. There are a large number of infiltration of inflammatory cells in interstitial, primary structure damaged, inflammatory response significantly. The clinical results were determined with double blind control method.

### 1.8 Statistical analysis

Statistical methods used Ridit analysis. The test level  $\alpha=0.05$ .

## 2 The results

### 2.1 The expression of HSP70

Ridit analysis of the HSP70 expression at first days, fourth days between the experiment group and the control group>0.05, differences were no significant. Ridit analysis of the HSP70 at seventh days between experimental group and the control group,  $R_e=0.6319$ ,  $R_c=0.3681$ ,  $u=2.89$ ,  $P<0.01$ , the difference was significant. According to the two sets of R values it was thought that the HSP70 expression of the experimental group in the seventh day more than the control group (table 1).

### 2.2 The intensity of rejection

The experimental group were all acute rejection occurred, while in the control group no acute rejection occurred. Ridit analysis of the HSP70 expression and small bowel immune rejection classification of the experimental group at first days and fourth days

showed differences were no significant>0.05. Compared with the seventh days,  $R_{light}=0.7167$ ,  $R_{middle}=0.7654$ ,  $R_{sever}=0.9568$ ,  $X^2=33.97$ ,  $P<0.01$ , the difference was significant. According to the R values in each group that high expression in the experimental group with highly HSP70 expressed in the seventh day small bowel immune rejection was serious.

**Table 1** the experiment group and the control group of HSP70 expressed in rat intestine 1, 4, 7 days after operation

group	the 1st day			the 2st day			the 3st day						
	—	+	+++	—	+	+++	—	+	+++				
experiment group		1	4	7	8	2	2	4	12	1	1	8	10
control group		1	3	9	7	4	8	4	4	3	6	9	2

**Table 2** the experimental group HSP70 expression and small bowel immune rejection grade comparison

grade	the 1st day			the 2st day			the 3st day					
	—	+	+++	—	+	+++	—	+	+++			
Mild rejection	0	1	2	3	1	1	1	4	1	0	1	1
Moderate rejection	0	2	1	3	0	1	2	3	0	1	3	2
Severe rejection	1	1	4	2	1	0	1	5	0	0	4	7

## 3 Discussion

Heat shock protein is a general term for a large family of proteins, named it since Ritossa and Tissieres first found it can synthesize in the super normal temperature environment<sup>[3]</sup>. Recent studies have confirmed that HSP and immune response have extensive connections, with the function immune regulation and activation of other cytokines.

12ng peptide HSP (including HSP 70 and gp96,) can start a strong immune response.HSP specific start MHC-I restricted T cell or CD8+T cell response, and MHC-I antigen binding to MHC-I molecules is a process rely on the HSP switching .HSP carries a peptide binding to macrophage surface receptors, then via the macrophage self class I molecules presenting sensitized cytotoxic Tlymphocytes.

Generally, HSP through the following ways to participate in antigen presenting process<sup>[4]</sup>: endogenous antigen hydrolyzed by protease in the cytoplasm, In the participation of HSP70 and HSP90, peptides formed by hydrolysis transport into the endoplasmic reticulum with the help of polypeptide transporter (TAP), submitted by gp96 or trimmed after submitted to the newly synthesized class MHC-I molecules, forming the peptide -MHC-I complex, and then transports to the cell surface, for CD8+T cells Identification of TCR.In exogenous antigen presentation, when the antigen presenting cells (APC) intake of exogenous antigen, can induce a large number of the HSP70 family members in cells:

72/74kD peptide binding protein (PBP72/74), which combine with the antigen and ATP, making antigen dissociate peptide fragment, then antigen peptide will be passed to the MHC-II molecule by the PBP72/74, expressing to cell membrane, presenting to the immune active cells for the immune response.

Our experiment shows, Seventh days after operation, the HSP70 expression of allogeneic transplantation group was higher than that of isograft group; the small bowel immune rejection was severer with higher expression of HSP70. Speculated that in the organ transplant process, the small intestine due to ischemia, hypoxia, injury induced HSP70 production, while this process produced HSP70 have the functions of synergistic immune, the recruitment and activation of T lymphocytes, causing a strong immune response, The denatured protein and various cytokines released by damaged cells will further stimulate the graft synthesis of HSP70, thus forming a

vicious spiral, giving the grafts a new attack.

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