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Indirect Immunofluorescence Expression of ZO-1 and Pan-cadherin during Maturation of Hepatocytes and Biliary Network Formation in Mice

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Abstract

Background The tight junctional complexes had been demonstrated in hepatocytes and in both epithelium and endothelium. In multicellular organisms, cell-cell adhesion is critical for development and morphogenesis.

Objective To investigate the spatiotemporal organization of the immunohistochemical markers anti ZO-1 and anti Pan-cadherin during the prenatal development of the mice liver and to correlate the expression of these markers with histogenesis during hepatocyte maturation and biliary network formation.

Methods Forty eight (48) pregnant mice were scarified at subsequent gestational days from day 14 till the first day of postnatal life. Paraffin blocks and sections of the liver extracted from the embryos obtained from these pregnant mice and from neonates at the first postnatal day of life were prepared. The sections were stained using the anti ZO-1 and anti Pan-cadherin immunohistochemical markers.

Results The sequential steps of anti ZO-1 indirect immunofluorescence reactivity in the developing liver tissue showed chronological variability, during days 14, 15, and 16; positive cell surface labeling was weaker adjacent to the blood vessels. The next step includes anti ZO-1 reactivity in late prenatal and postnatal liver tissue showing marked uniform reactivity. The sequential steps were also demonstrated in the anti Pan-cadherin immunohistochemical reactivity. The reactivity of the liver tissue during days 14, 15, and 16 showed disregarded cell surface labeling that increased markedly in later prenatal and postnatal periods.

Conclusion The initial stages in the development include the undifferentiated specification the hepatic lineage characterized by regional establishment of zonula occludens in the developing liver cells and paucity in the development of the zona adherens complexes. The later stages showed histological and functional maturation reflected by anti ZO-1 and anti Pan-cadherin reactivity that represent a requirement for the physiological functions in the entire liver tissue.

Keywords Embryo, mice, Zo-1, Pan-cadherin, immunhistochemistry.

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List of abbreviation: FITC= Fluorescein isothiocyanate, IgG=Immunoglobin G, IHC= Immunohistochemistry, TR= Texas Red, ZO-1=zonulaoccludans.

Introduction

Several studies used mice for research on liver structures and functions assumed that there is similarities between the humans, mouse and rat liver [1]. In mice, the development of the liver is the same that in

human by formation of hepatocytes and biliary epithelial cells that are derived from the endodermal germ layer, while the stromal, stellate, kuppfer cells and blood vessels all are of mesodermal germ layer [2]. A mouse liver extent the whole sub-diaphragmatic space and divided into four lobes (right, medial, left, and caudate lobes) [3].

The junctional complexes are intercellular connection that are of three major types; vertebrates, tight junctions, adherens junctions (including zonulaadherens and macula adherens) and gap junctions [4]. The bile canaliculi between liver cells are split from the intercellular space by the tight junctions, which forming a barrier to large molecule and substance exchanging between the biliary section and the blood section [5]. The zonula occludans-1 (ZO-1) has been verified in hepatocytes of liver in both epithelium and endothelium [6]. The procedure for cell-to-cell adherens junctions has been illustrated in rat liver [7]. The gap junctions occur between liver cells at patchy distances from the bile canaliculi [8].

Pan-cadherin is a termed of antibodies that detect cadherin proteins in a variety of tissue organs. The Cadherin is a polypeptides (about 720–750 amino acids long) that part of super family of trans-membrane glycoproteins [9].

This study was designed to investigate the spatiotemporal organization of the immunohistochemical markers (Anti ZO-1 and Pan-cadherin) during the prenatal development of the mice liver till the 1st day of parturition.

The expression of these markers would be correlated with histogenesis during hepatocyte maturation and biliary network formation.

Methods

Forty-eight (48) adult femalespregnant mice aged about 10-12 weeks, weighing between 25-30 g, apparently active and healthy had been used in this study. These mice were divided according to the days of pregnancy to (day 14,15,16,17,18,19,20 and postnatal "1st day after parturition"), and select six (6) mice for each of these days. The number of embryos (or newborns) obtained ranged from (6-8) from each mouse. Two (2) of these embryos (or newborns) were taken randomly from each mouse. The total number of embryos was (12) for each day. The liver embryos were fixed in 10% formalin for 48 hours and paraffin blocks were prepared [10]. Sections of 5 µm thickness were cut using the electrical microtome (Richert - Jung, 2030 MOT Biocut).

Two immunohistochemistry (IHC) markers had been used in this study from (SANTCRUZ BIOTECHNOLOGY and US BIOLOGICAL):

1- Monoclonal, Anti ZO-1 (R40.76) (primary).
   - Goat anti-rat IgG-FITC: sc2011 (secondary).
2- Monoclonal, Anti Pan-Cadherin, (primary).
   - Goat anti-mouse IgG-TR: sc-2781 (secondary).

Images of Anti ZO1 and Anti Pan-cadherin were captured by Zed Axis camera (5 mega pixels) placed directly over the head of the fluorescent microscope linked with a desktop computer to saved images.

Results

Indirect immunofluorescence reactivity of ZO-1

Section of the adult rat kidney was used as a positive control of the anti ZO-1 IHC reactivity. Sections of embryos in each day were used as a negative control by stained only the secondary of ZO-1.

During 14, 15, 16 days

The paraffin sections of the developing hepatic parenchyma during the 14th, 15th and 16th days of gestation showed positive IHC reactivity to anti ZO-1 antibody. The endothelial cells of the hepatic sinusoids showed very weak reactivity at this developmental stage. The fluorescent IHC reactivity appeared to be fainter at the peri-sinosoidal hepatic parenchymal tissue. The variable cellular elements of the liver tissues could not been distinguished as the histological criteria of the tissue are not demonstrated using the IHC labeling. The cell surface

immunoreactivity verifies variability of the cell-size at high power field. The size variability ranged from small, intermediate, to large cell size. The small size immunoreactive cells are arranged in a linear-chain like architecture all over the field. The cellular reactivity showing the intermediate and large size of cells is less numerous in comparisons to the small size cells. The cell surface IHC fluorescence reactivity appears more intense around the larger cell (Figure 1).

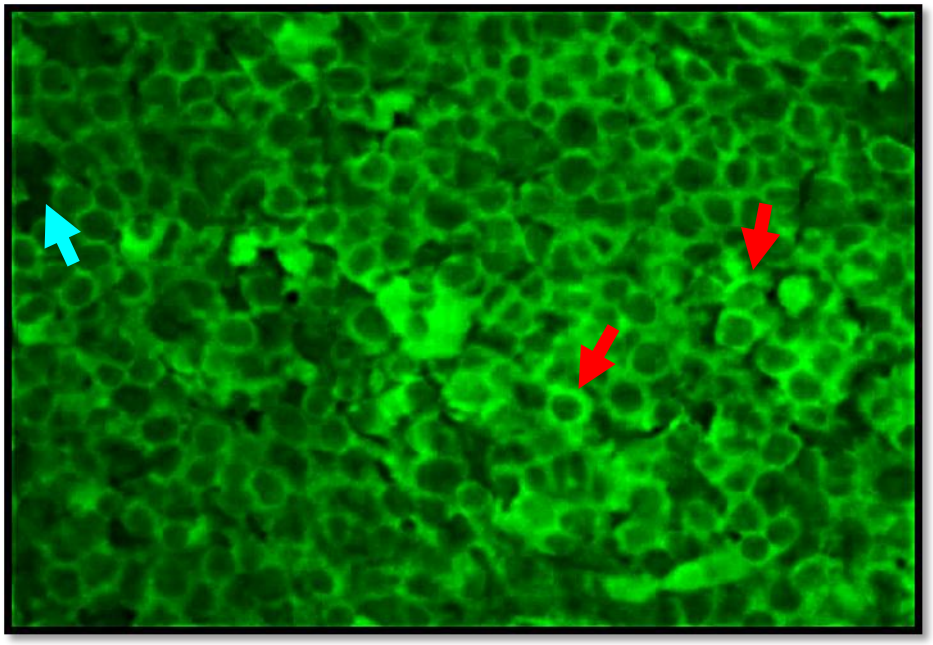

Figure 1. The section of mouse liver embryo at the day 15 showed positive reactivity to anti ZO-1 antibody in the cell surface by using indirect immunoflourescence (Red arrow), hepatic vessel (Blue arrow). 100X

During 17th and 18th days

The vascular endothelial cell showed weak reactivity, the vascular lumen contained many strongly fluorescent red blood cells. The IHC reactivity showed uniform intensity in all parts of the liver parenchyma. The immunoreactivity on the cell surface showed mostly large and intermediate size cells (Figure 2).

During days 19 - to - postnatal (1st day after parturition)

The fluorescence reactivity all over the section showed distinguished cell surface binding demarcating the cellular boundaries. Other cellular element showed the same pattern of reactivity seen in the previous stages (Figure 3).

Indirect immunofluorescence reactivity of Pan-Cadherin

Section of the adult rat kidney was used as a positive control of the anti Pan-cadherin IHC reactivity. Sections of embryos in each day were used as a negative control by stained only the secondary of Pan-Cadherin.

During days 14, 15 and 16:

The Pan-cadherin IHC reactivity was very weak. No regional variability was seen in different parts of the sections. The intravascular red blood cells showed strong fluorescent reactivity. The ghost-like fluorescence reactivity does not allow clear examination of the cellular outlines (Figure 4).

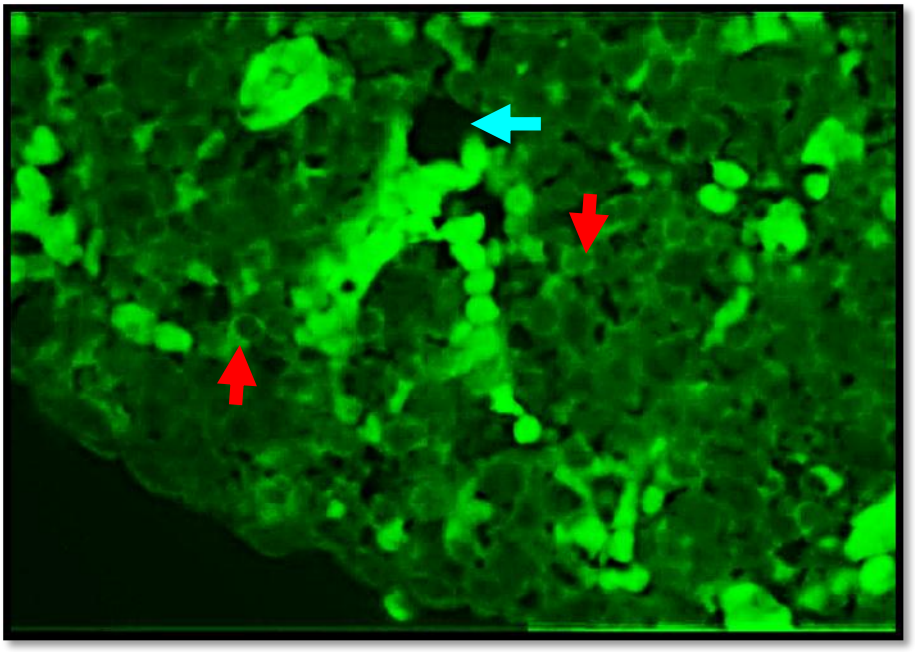

Figure 2. The section of mouse liver embryo at the day 18 showed positive reactivity to anti ZO-1 antibody in the cell surface by using indirect immunoflourescence (Red arrow), hepatic vessel (Blue arrow). 100X

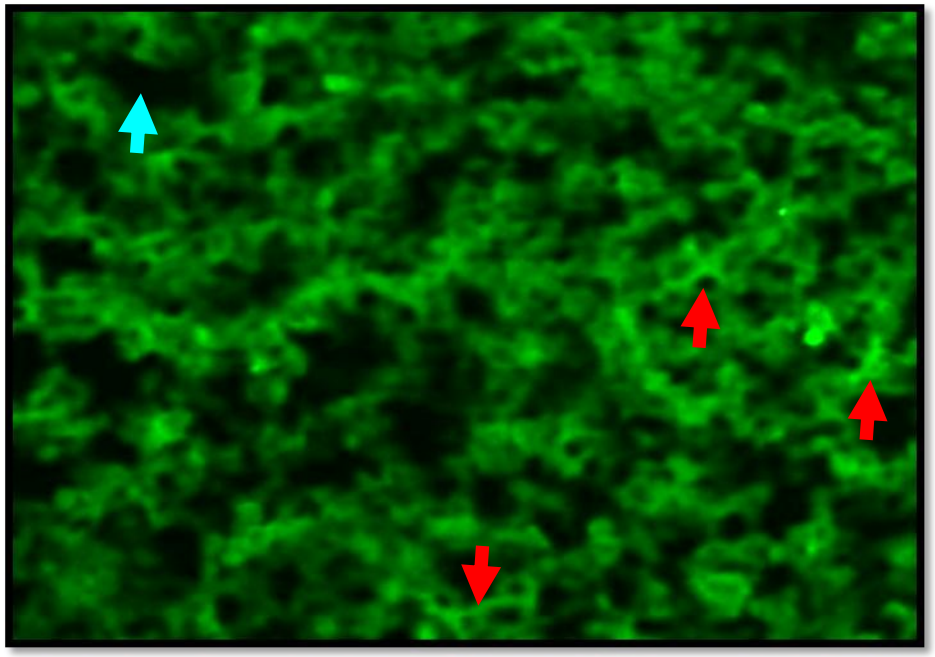

Figure 3. The section of mouse liver embryo at theday 20 showed positive reactivity to anti ZO-1 antibody in the cell surface by using indirect immunoflourescence (Red arrow), sinusoid (Blue arrow). 100X

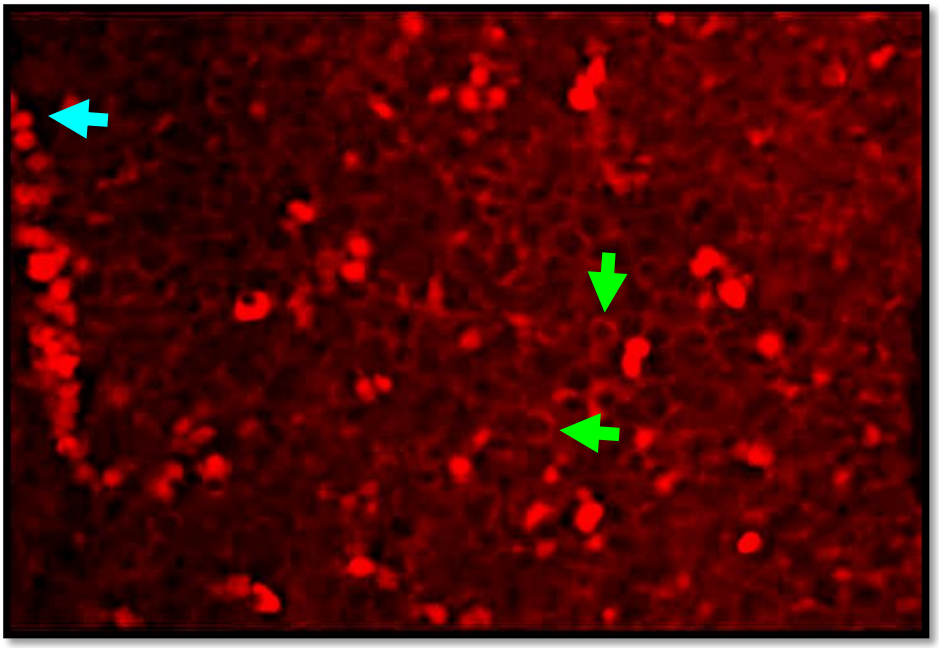

Figure 4. The section of mouse liver embryo at the day 15 showed positive reactivity to anti Pan-cadherin antibody in the cell surface by using indirect immunoflourescence (Green arrow), hepatic vessel (Blue arrow). 100X

During days 17- to - postnatal (1st day after parturition)

The IHC reactivity of the pancadherin in the paraffin sections showed obvious fluorescence in all part of the sections. The outline of the cellular architecture showed large and

The liver develops in a sequential range of steps that are regulated by (intrinsic mechanisms) and (extracelluar signals) leading in differentiation of liver paranchyme (11).

The tight junctional complexes had been demonstrated in the liver cells, and the cell-cell adhesion was confirmed to have a critical role during morphogenesis (12).

These actualities were supported by the results of the IHC descriptions of this study, which suggest the formation of junctional complexes as an important cellular apparatus involved in spread of signaling regulating the maturation of the hepatic tissues. The developmental peroid involved in this study covers the duration of hepatocyte maturation and billiary

intermediate cell size, simulating the cellular pattern of immunohistochemical reactivity observed in the similar stages labeled with anti ZO-1 antibody (Figure 5).

Discussion

network formation extending between gestational day 14 till the 1st day of postnatal life.

The sequential steps of anti ZO-1 IHC reactivity in the developing liver tissue showed chronological variability, during days 14, 15, and 16 positive cell surface labeling was seen, which was weaker adjacent of the blood vessels. The outline of cell surface reactivity distinguished variable cell size ranging from small to large cells. The next step includes anti ZO-1 reactivity in late prenatal and postnatal liver tissue showing marked uniform reactivity.

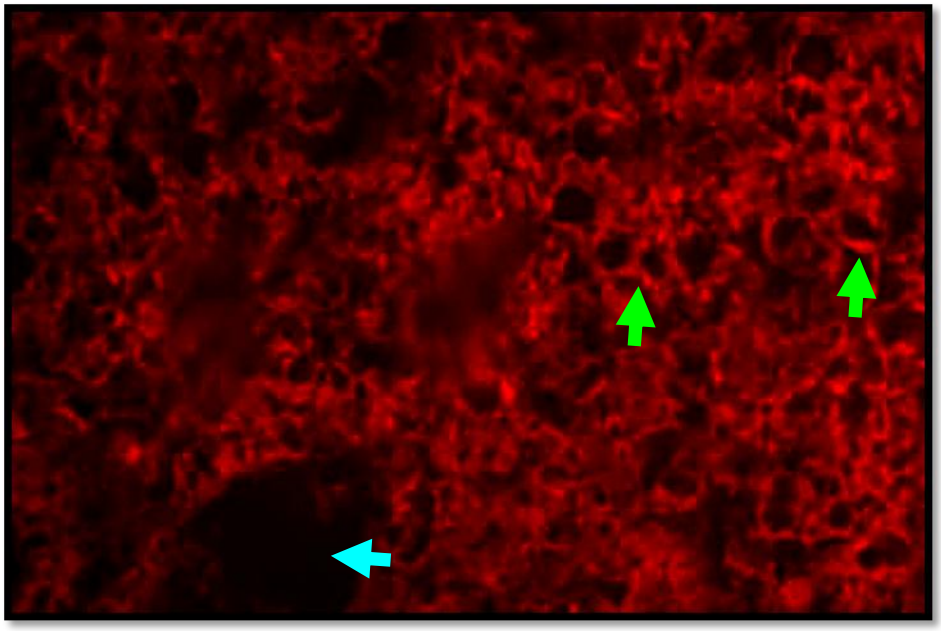

Figure 5. The section of mouse liver embryo at the day 20 showed positive reactivity to anti Pan-cadherin antibody in the cell surface by using indirect immunoflourescence (Green arrow), hepatic vessel (Blue arrow). 100X

The IHC reactivity of ZO-1 during day 14, 15, 16 days described in this study showed very weak reactivity in the endothelial cell of the hepatic sinusoids at these early developmental stages. This finding supported by the finding of (Sheridan, 1966) suggesting that minute tight junctions observed within the primitive tissues of the young chick embryo represent the sites of lowered cell to cell electrical resistance, which have been reported by Sheridan [13].

Potter et al. [14] had discovered that electrical coupling between developing cells occurs prior to cell differentiation in embryonic development. Also, preliminary report done by Sheridan [13] demonstrated low electrical resistance between cells in chick embryos and other developing vertebrate. These reported are in agreement with conclusion of the ZO-1 IHC reactivity investigated in this study.

The sequential steps were also demonstrated in the anti Pan-cadherin IHC reactivity, disregarded cell surface labeling of the liver tissue was seen during days 14, 15, and 16 that will have increased markedly in late prenatal and postnatal periods.

Accordingly, the development of junctional complexes during histogenesis of the liver tissue can be divided into distinct stages based on the IHC reactivities of markers involved in this study.

The initial stages in the development include the undifferentiated specification the hepatic lineage characterized by regional establishment of zonula occludens in the developing liver cells and paucity in the development of the zona adherens complexes.

From the results of this study, it was concluded that later stages showed histological and functional maturation required for the physiological value of these junctional complexes in the entire liver tissue.

This conclusion is in agreement with descriptions of Zaret [15] that reported detection of liver-specific markers in mouse since days 8-9, the hepatic paranchyma at this stage possess the potential to differentiate into both hepatocytes and bile duct epithelial cells [16].

The zona occludens and zona adherens are recommended for intercellular communications [5]. The signaling control of the

liver development includes many of the fibroblast growth factors, which displaying hepatogenic properties in many embryonic species [17]. The dynamics of signaling controlling hepatic specification in mouse embryo was also established by [18]. The surfacing of the junctional complexes in association with maturation of the liver cells could play role in transmitting intercellular signaling exhibiting the hepatogenic properties. It was stated that hepatic cells maturated when they switch from a hematopoietic microenvironment to a metabolic organ [19], then after the growth of liver cells arrested during the postnatal development [20]. The postnatal differentiation of the hepatocytes and other non-parenchymal cells to creates the organized liver lobules that are the basic units of the liver tissue. This report could be implicated for the rationality of choosing the limitation of the first postnatal day as the latest stage involved this study.

The endodermal cells committed to the hepatic cells lineage undergo maturation programs to acquire various specific metablolic functions and differentiate into either hepatocytes or bile duct cells. The previous suggestions reported that the embryonic hepatic cells should not simply be considered as immature nonfunctional cells [21].

This suggestion could interpret the pattern of IHC reactivity in this study that show equivalent binding in both late prenatal and postnatal stages and establishing evidences that the late prenatal and the postnatal hepatic paranchyma achieve the property of specific maturation.

This study concluded:

1. The immunehistochemical results represent a step to elaborate the mechanism underlying the fate of hepatoblast differentiating into hepatocytes or biliary epithelial cells.
2. The histology of the developing liver provides a framework for interpretation the immunohistochemical investigations of liver tissue organization.
3. The histology of developing liver supported reports that the liver tissue from day 19 till the early postnatal days showed no marked morphological changed.
4. The Anti Pan-Cadherin reactivity showing disregarded labeling of the liver tissue in younger embryo.
5. The junctional complexes were divided into distinct stages. The initial stage charactrized by regional establishment of zonula occludens and paucity of the zona adherens. The later stages showed histological and functional maturation that require the physiological value of junctional complexes in the entire liver tissue.
6. Equivalent immunhistochemical reactivity in both late prenatal and postnatal stages indicating specific maturation.
7. The mosaic Anti ZO-1 reactivity gives insights on the maturation pattern of hepatocytes which depend on cell-cell and cell-matrix interactions controlled by adhesion molecules and intercellular junctions.
8. Uniform Anti Pan-Cadherin reactivity at late prenatal and postnatal days indicate normal tissue integrity as cadherins regulates morphogenesis in the liver during development, this functional criterion was associated with maturation of the liver tissue

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Author contributions:

Dr. Ali: the M.Sc. candidate performing the laboratory research work and performing production of the results. Dr. Mubarak: the supervisor of the MSc research performing the interpretation of the results.

Conflict of interest

The authors disclose no any financial and personal relationships with other people or organizations that inappropriately influence (bias) this work.

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