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Minireview

Intermediate trophoblast—A distinctive, unique and often unrecognized population of trophoblastic cells

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ABSTRACT

The trophoblast forms an outer layer of the blastocyst in the developing placenta and fetal membrane chorion. It is composed of different types of cells. Two main cell types are cytotrophoblasts and syncytiotrophoblasts. The third type of trophoblastic cells, often "forgotten" in most of histological and embryological textbooks, is morphologically and functionally between the first and second one, therefore, it is called the intermediate trophoblast. There is no mention of it in the internationally accepted *Terminologia Embryologica*. This term is not universally used by pathologists as some of them prefer the name extravillous trophoblast. This review provides an overview of morphology, localization, function and immunohistochemistry of different types of intermediate trophoblast cells. An indisputable reason for categorizing these cells as a distinct group is the fact that they are a source of various forms of gestational trophoblastic disease.

1. Introduction

The trophoblast is a very interesting structure from an embryological and also histopathological point of view. The development of the human embryo progresses rapidly immediately after implantation, and the trophoblast enables the embryo to adhere to the uterine epithelium, facilitating embedding into the endometrial wall and forming the future fetal part of placenta. The study of the trophoblastic cell invasion into the uterus wall (Liao et al., 2012; Fu et al., 2013) or study of the regulation of the growth of placental villi covered by trophoblast (Kučera et al., 2010; Jirkovská et al., 2012), both under physiological and pathological conditions, are only two examples of many mechanisms, which are the objects of interest of many researchers.

Trophoblast (from Greek words *trophé* – nourishment, food; *blastós* – sprout) is composed of different subpopulations of cells. Cytotrophoblast represents trophoblastic stem cells whereas syncytiotrophoblast is the terminally differentiated multinucleated cell mass ("*syncytium*") that produces most of pregnancy-associated hormones (including human placental lactogen and human chorionic gonadotropin) and regulates the diffusion of oxygen, carbon dioxide, nutrients and waste products between mother and embryo/fetus (Shih and Kurman, 2001; Kurman and Shih, 2014). The layer of syncytiotrophoblast is probably derived from an underlining layer of mononuclear cytotrophoblast, but the process of trophoblast cell fusion is poorly understood (Jaggi et al., 1997; Ji et al., 2013). In recent years, mostly in pathological and gynecological literature, the term "intermediate trophoblast" has been introduced (the historical contexts of this scientific discovery has been reviewed by Kurman and Shih, 2014). The intermediate trophoblast is a distinct type of trophoblast that shares some of the morphologic and functional features of both cytotrophoblast and syncytiotrophoblast. Physiologically is seen in the normal implantation site, at the anchoring villi (termed "*implantation site intermediate trophoblast*" or "*villous intermediate trophoblast*") or in the chorionic laeve of the fetal membranes (designated as "*chorionic-type intermediate trophoblast*"). Despite the fact that, after entering the term "intermediate trophoblast" in the database Medline/PubMed, more than 150 different articles appear, there is no mention about it in internationally accepted *Terminologia Embryologica* (FIPAT, 2013). *Terminologia Embryologica* is a standardized list of words used in the description of embryonic and fetal structures produced by the *Federative International Committee on Anatomical Terminology* on behalf of the *International Federation of Associations of Anatomists*. The terminology of the trophoblast also varies in different internationally accepted embryological

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textbooks; most of them, e.g. Cochard (2012), Dye (2012), Sadler (2012), Carlson (2014), Dudek (2014), Schoenwolf et al. (2015) or Moore et al. (2016) contain no mention of "intermediate trophoblast".

On the other hand, the term "intermediate trophoblast" is commonly used by pathologists for various reasons. It is a cell group with characteristic morphological features, immunohistochemical markers and different functions. This group of cells is also a source of various forms of trophoblastic diseases, e.g., placental site nodule (Shih et al., 1999; Pramanick et al., 2014), placental site trophoblastic tumor (Piura, 2006), epithelioid trophoblastic tumor or exaggerated placental site (Genest et al., 2003). In general, gestational trophoblastic disease represents a clinically and histopathologically heterogeneous group of diseases and is a rare complication of pregnancy. Gestational trophoblastic diseases are characterized by abnormal growth of different cell types of the placental trophoblast, including intermediate trophoblast (Horn et al., 2009, 2014). However, some studies predict other important roles of intermediate trophoblast in the pathogenesis of some pregnancy-associated complications. According to Liu et al. (2004), the altered function of intermediate trophoblast during trophoblast invasion (associated with the lower expression of melanoma cell adhesion molecule) also play a role in the pathogenesis of pre-eclampsia. Papamitsou et al. (2011) discovered that a lower expression of progesterone receptor A on the surface of the intermediate trophoblastic cells is correlated with higher risk of miscarriage of the implanted and growing embryo/fetus.

In this review we would like to contribute to completion of the histological and embryological terminology similarly to the development of the anatomical terminology from the year 1895 when the first Latin anatomical nomenclature was published as *Basiliensia Nomina Anatomica* (Kachlik et al., 2008).

2. Morphology of the trophoblast

The trophoblast forms an outer layer of the blastocyst and is a basis for the developing placenta and fetal membrane chorion. Based on their anatomic location, morphological features and immunohistochemical markers, several trophoblastic subpopulations in human placenta and fetal membranes have been recognized and defined. The trophoblast is composed of three different cell types – cytotrophoblast, syncytiotrophoblast and intermediate trophoblast. From a pathological point of view, they vary not only morphologically and functionally, but also give rise to different diseases commonly termed as gestational trophoblastic disease (Shih and Kurman, 2001; Mao et al., 2007; Shih et al., 2011).

The trophoblast on the surface of the chorionic villi is termed villous trophoblast and is composed mostly of cytotrophoblast and syncytiotrophoblast cells. The intermediate trophoblast forms only small part of the villous trophoblast. The intermediate trophoblast on the villous surface forms trophoblastic columns that anchor the fetal part of placenta to the implantation site. In all other locations, the trophoblast is referred to as extravillous trophoblast and is composed mostly of intermediate trophoblast. The extravillous trophoblast infiltrates the decidua, myometrium and spiral arteries of the placental site (Shih and Kurman, 2001; Kurman and Shih, 2014) and is also often termed endovascular trophoblast (Frank and Kaufmann, 2006). The identification of extravillous trophoblast in routinely stained tissue sections (such as hematoxylin and eosin staining) is hardly possible. Antibodies to cytokeratins are stated to be useful markers for discrimination between extravillous trophoblast and decidual cells; beyond this they are also useful in distinguishing intra-arterial trophoblast from maternal endothelium (Moser et al., 2011; Enders and Blankenship, 2012).

2.1. Cytotrophoblast

The cytotrophoblast (so-called Langhans cells) consists of small uniform polygonal to oval cells with distinct cell borders and forms an inner layer on the surface of the chorionic villi. Over half of the cytoplasm of cytotrophoblastic cell is occupied by the single round to oval nucleus (Fig. 1a). The cytoplasm is clear or granular at the light microscopic level; at the electron microscopic level several polyribosomes and elongated mitochondria are recognizable (Jaggi et al., 1997; Fox and Sebire, 2007). The cytotrophoblast does not produce hormones, therefore it is immunohistochemically negative for placental hormones, but is positive for cytokeratins. The cytotrophoblast represents stem cells. The proliferating index Ki-67 is 25–50%, being highest in the early stage of pregnancy; the decreasing with increasing gestational age (Fig. 1b). These mitotically active cells are able to differentiate along two pathways – to syncytiotrophoblast and intermediate trophoblast (Shih and Kurman, 2001). On the villous surface, the cytotrophoblast fuses directly to form syncytiotrophoblast (Ji et al., 2013). The differentiation of cytotrophoblast in extravillous areas into intermediate trophoblast is accompanied by a decrease in or loss of proliferative activity (Shih and Kurman, 1998).

2.2. Syncytiotrophoblast

The first pathway of cytotrophoblast differentiation means direct fusion of mononuclear cells on the surface of placental villi into multinucleated large cells of syncytiotrophoblast. Syncytiotrophoblast has abundant eosinophilic to amphophilic vacuolated cytoplasm and multiple dark, often pyknotic nuclei. It overlies cytotrophoblast on the chorionic villi (Fig. 1a). Syncytiotrophoblast cells are terminally differentiated without mitotic activity. At the electron microscopic level, the cytoplasm of the syncytiotrophoblast has a translucent appearance, with granular endoplasmic reticulum and few dense granules. The apical surface creates a large number of elongated microvilli that considerably increase the surface area and the subapical cytoplasm contains several pinocytotic vesicles (Jaggi et al., 1997). Syncytiotrophoblast is in direct contact with maternal blood and is responsible for respiratory gases, nourishment and waste substance exchange between the mother and fetus as well as to exhibiting a required degree of immune tolerance. One of the main functions of syncytiotrophoblast is also the production of most of the placental hormones (Wang and Zhao, 2010). The most important are human chorionic gonadotropin (hCG), human placental lactogen (hPL), placental alkaline phosphatase (PLAP), estradiol, progesterone, placental growth hormone and inhibin-α (Fig. 1c). Immunohistochemically proliferating index Ki-67 is negative (Fig. 1b) and the intensity of pregnancy hormone positivity depends on the gestational week.

2.3. Intermediate trophoblast

The second pathway of differentiation of cytotrophoblast leads to formation of the intermediate trophoblast. These cells have partial morphological and functional similarity to both former groups of trophoblastic cells. The intermediate trophoblast represents a heterogeneous cell population with different features.

The villous intermediate trophoblast consists of cells larger than cytotrophoblast cells, with pale cytoplasm and a single uniform round nucleus (Fig. 1a). They are localized in trophoblastic columns of anchoring villi. These cells are Ki-67 positive (Fig. 1b) and negative for inhibin-α (Fig. 1c).

Villous intermediate trophoblastic cells are a source of extravillous intermediate trophoblast of implantation site and extravillous intermediate trophoblast of chorionic type (Shih et al., 2011).