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Immunolocalization of nestin, mesothelin and epithelial membrane antigen (EMA) in developing and adult serous membranes and mesotheliomas

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ABSTRACT

The spatial and temporal distribution of epithelial membrane antigen (EMA), mesothelin and nestin was immunohistochemically analyzed in developing and adult human serous membranes and mesotheliomas in order to detect possible differences in the course of mesenchymal to epithelial transformation, which is associated with differentiation of mesothelial cells during normal development and tumorigenesis. Pleura and pericardium developing from the visceral mesoderm gradually transform into mesothelial cells and connective tissue. EMA appeared in mesothelium of both serous membranes during the early fetal period, whereas during further development, EMA expression was retained only in the pericardial mesothelium. It increased in both pleural mesothelium and connective tissue. Mesothelin appeared first in pericardial submesothelial cells and later in surface mesothelium, while in pleura it was immediately localized in mesothelium. In adult serous membranes, EMA and mesothelin were predominantly expressed in mesothelium. Nestin never appeared in mesothelium, but in connective tissues and myocardial cells and subsequently decreased during development, apart from in the walls of blood vessels. Mesothelial cells in the two serous membranes developed in two separate developmental pathways. We speculate that submesothelial pericardial and mesothelial pleural cells might belong to a population of stem cells. In epithelioid mesotheliomas, 13% of cells expressed nestin, 39% EMA and 7% mesothelin.

Introduction

The mesothelium lines the peritoneal, pleural and pericardial cavities, with visceral and parietal surfaces covering the internal organs and body wall, respectively. It comprises a monolayer of epithelial-like cells resting on a thin basal lamina supported by sub-serosal connective tissue containing blood vessels, lymphatic vessels, resident inflammatory cells and fibroblast-like cells (Wang, 1974; Ishihara et al., 1980; Albertine et al., 1982). During human development, the intraembryonic mesoderm divides into two layers, the splanchnic or visceral layer and the somatic or parietal layer. A continuous mesothelial membrane lines the margin of these two layers e.g., the entire intraembryonic celom, which is sub-divided into the pericardial cavity, two pleural cavities and a peritoneal cavity. In this phase of development, the mesothelial and submesothelial layers of the celom are referred to as the pericardium, pleura, and peritoneum respectively, and overall as serous membranes (reviewed by Thors and Drukker, 1997). Mesothelial cells are therefore derived from a primitive mesodermal origin, but share characteristics of both epithelial and mesenchymal cells (Whitaker et al., 1992). Thus, mesothelial cells differentiate during the process of mesenchymal to epithelial transformation, as previously shown for the meningeal membranes (Herrick and Mutsaers, 2004; Petricevic et al., 2010). Morphologically, mesothelial cells are considered in general to be similar at different serosal sites and also between different mammalian species (Baradi and Rao, 1976; Whitaker et al., 1980, 1982a,b). In their fully differentiated state, they form a monolayer of predominantly squamous cells approximately 25 μm in diameter, with characteristic surface microvilli and occasional cilia.

Mesothelial cells display many epithelial characteristics which include a polygonal cell shape, cytokeratin intermediate filaments (cytokeratins 6, 8, 18 and 19) (Czernobilsky et al., 1985), and the ability to form a basal lamina. However, they also show features of mesenchymal cells such as the presence of vimentin, desmin and upon stimulation, alpha smooth muscle actin (Afify et al.,

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2002). During mesenchymal to epithelial transformation, mesothelial cells retain some features of mesenchymal cells, thus combining features of both cell types, mesenchymal and epithelial. Possible functions of mesothelium include transport of fluids across serosal membranes, participation in serosal repair and inflammation, and protection from infection and tumor dissemination (Mutsaers, 2002). However, the cells that differentiate on the surfaces of serous and meningeal membranes are of different origin: in the head region neural crest cells (cells of neuroectodermal origin) participate significantly in the formation of leptomeninges, however, this is not the case for the typical serous membranes such as pleura and pericardium.

Tumors of mesothelial cells, known as mesotheliomas, are predominantly aggressive tumors of adults. The World Health Organization (WHO) recommends the terminology diffuse malignant mesothelioma when referring to malignant neoplasms arising from mesothelial cells. Several histopathological types of mesothelioma have been described. *Epithelioid mesothelioma*, has an epithelioid morphology, usually consisting of rather bland cells with abundant eosinophilic cytoplasm. *Sarcomatoid mesothelioma* is composed of spindle cells that have haphazard distribution. Some cases resemble fibrosarcoma, and others have a pattern resembling undifferentiated pleomorphic sarcoma. *Desmoplastic mesothelioma* consist of scattered atypical cells in a storiform or non-specific pattern set in a dense collagenous background in more than 50% of cases of the tumor. *Biphasic mesothelioma* contains a mixture of the other patterns, in most cases a combination of the epithelioid and sarcomatous patterns. By definition, each component should comprise at least 10% of the tumor (Travis et al., 2004).

Mesothelin is a differentiation antigen that was originally described as the antigenic target of the K1 monoclonal antibody (Chang et al., 1992a,c), and was generated using the OVCAR-3 ovarian carcinoma cell line (Chang and Pastan, 1996). The secreted form is identical to the megakaryocyte-potentiating factor (Kojima et al., 1995). It is, however, the GPI-linked membrane form that has generated the most interest as a potential immunohistochemical marker in tumor diagnosis (Argani et al., 2001). Because mesothelin is strongly expressed in normal mesothelial cells and in mesotheliomas, this protein has been used as an immunohistochemical marker to distinguish between epithelioid mesotheliomas and pulmonary adenocarcinomas (Chang et al., 1992a,c). It is also expressed in meningiomas, pancreatic adenocarcinomas, and squamous cell carcinomas of the head, neck, lung, esophagus, cervix and vulva (Chang et al., 1992b; Chang and Pastan, 1996; Frierson et al., 2003; Johnson et al., 2008; Petricevic et al., 2010). It is believed that mesothelin is a cell surface differentiation protein possibly involved in cell adhesion (Chang and Pastan, 1996). Although mesothelin was shown during the development of human meninges and in meningiomas (Petricevic et al., 2010), its expression in human embryonic and fetal serous membranes has so far not been studied.

Nestin is one of the intermediate filaments, together with vimentin and glial fibrillary acidic protein (GFAP), and is detected abundantly in neuroepithelial stem/progenitor cells in the developing central nervous system of embryonic rats and humans (Lendahl et al., 1990; Tohyama et al., 1992, 1993; Messam et al., 2000). Nestin forms intermediate filament bundles, perhaps with vimentin, by copolymerization in neuroepithelial cells (Eliasson et al., 1999; Rutka et al., 1999). It is rarely detected in non-neoplastic brain tissues, occurring sometimes faintly in vascular endothelial cells. During human development, nestin was detected in the developing optic nerve (Bozanic et al., 2006), and spinal ganglia (Vukojevic et al., 2009). It has been detected in human gliomas, glioblastomas, hemangioblastomas and meningeomas (Dahlstrand et al., 1992; Sugawara et al., 2002), and also in endothelial cells in active proliferation (Sugawara et al., 2002.). Nestin was found in developing human meninges in some arachnoidal cells and mostly in vascular endothelial cells (Petricevic et al., 2010).

Table 1
Age and number of human embryos and fetuses analyzed in the study.

Age (weeks)	CRL (mm)	Carnegie stage	FL (mm)	No.
7–8	23	21	–	3
9–10	57	–	8	2
15–16	142	–	29	2
20–22	214	–	45	2

CRL, crown-rump length; FL, foot length.

Epithelial membrane antigen (EMA) belongs to a heterogeneous family of highly glycosylated transmembrane proteins known as human milk fat globule (HMFG) membrane proteins. This family of antigens is found in secretory and in non-secretory epithelial cells (e.g., squamous epithelium), but rarely in non-epithelial cells. EMA stains normal epithelium, the apical portion of duct lining cells in mammary and other glandular epithelia, arachnoidal cells of human prenatal and postnatal leptomeninges, squamous epithelium (patchy), adenocarcinomas from a variety of sites, squamous and transitional cell carcinomas, small cell anaplastic carcinomas, mesotheliomas, meningeomas, histiocytic lymphomas, and synovial and epithelioid sarcomas (Heyderman et al., 1979; Cordell et al., 1985; Pinkus and Kurtin, 1985; Petricevic et al., 2010). To date, the expression of EMA in embryonic, fetal and postnatal human serous membranes has not been studied.

In this study, patterns of appearance of EMA, mesothelin and nestin were analyzed in developing and adult human pleura and pericardium, as well as in epithelioid mesotheliomas. During the course of mesenchymal to epithelial transformation and differentiation of the surface mesothelial cells, differences between the two serous membranes appeared in temporal and spatial distribution of the three markers in the mesothelium, which implies differences in their origin and developmental pathways. In mesotheliomas, the same cell populations were randomly scattered, thus indicating that disturbances in the process of mesenchymal to epithelial transformation might participate in tumorigenesis.

Materials and methods

Human material

A total of 9 human concepti between developmental weeks 7 and 22 were collected after spontaneous abortions from the Department of Gynecology and Obstetrics, University Hospital Split, Croatia and after tubal pregnancies from the Department of Pathology, University Hospital Split. The embryos and fetuses were examined macroscopically and measured. Only normal concepti, lacking indications of abnormality, intrauterine death or macerations were used in our study. The embryonic tissues were treated as post-mortem material with permission of the Ethical and Drug Committee of the University Hospital Split, in accordance with the 1964 Helsinki Declaration. The postovulatory age was estimated on the basis of the menstrual data, correlated with crown-rump length (CRL) and Carnegie stages (O'Rahilly and Gardner, 1971; Table 1).

Serous membranes from the surfaces of heart and lungs (taken during autopsies) of 6 adult patients who died without cardiac and pulmonary lesions, and samples of 6 mesotheliomas were immunohistochemically analyzed with permission of the Ethical and Drug Committee of the University Hospital Split. The latter group included WHO diffuse malignant mesotheliomas, epithelioid subtype (Travis et al., 2004) collected between the years 2000 and 2009 at the University Hospital Split. Patients' characteristics for the serous membrane samples and tumor characteristics are listed in Tables 2 and 3, respectively.