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Apoptotic pathways in ovarian surface epithelium of human embryos during embryogenesis and carcinogenesis: Close relationship of developmental plasticity and neoplasm

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ABSTRACT

Cell differentiation and different pathways of cell death were immunohistochemically analyzed in ovaries of six human embryos, 20 serous borderline tumors (SBT) and ovarian serous carcinomas (OSC) using markers for apoptosis (caspase-3, AIF, TUNEL) and stemness (Oct-4). In the 5–8-week ovaries, caspase-3 was absent in the ovarian surface epithelium (ose) and mildly positive in the ovarian stroma (os), AIF was expressed moderately, while Oct-4 expression gradually decreased during that period. Some ovarian cells expressed only caspase-3 or AIF together with TUNEL, while both caspase-3 and AIF were co-expressed in other ovarian cells. Mild expression of Oct-4 and caspase-3 characterized some cells of SBT, while their expression varied from mild to strong in OSC. AIF displayed mild to strong expression in ose of SBT and moderate to strong expression in OSC, while no expression of AIF was observed in os of both tumors. In the ose of both SBT and OSC, caspase-3 and AIF were co-expressed only occasionally, while AIF and Oct-4 were co-expressed strongly. Our study showed the presence of stemness cells and different pathways of cell death (caspase-3 and AIF-mediated) in the ovarian tissue during development and carcinogenesis, indicating the correlation between developmental plasticity in human embryonic ovaries and OSC.

Introduction

Apoptosis is an evolutionarily conserved and genetically regulated form of programmed cell death, representing an important mode of genetically controlled elimination of cells, both in development and in many types of cancer (Elmore, 2007; Natarajan and Becker, 2012). The mechanism of programmed cell death is very well investigated in apoptosis, which displays two main apoptotic pathways: the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway (Igney and Krammer, 2002; Jiang and Wang, 2004). The intrinsic or mitochondrial apoptotic pathway is primarily initiated in response to cellular oxidative stress or DNA damage. It is mediated via p53 gene, which up-regulates proapoptotic and antiapoptotic factors of Bcl-2 family members, leading to conformational changes of Bax and formation of pores in the outer mitochondrial membrane (Elmore, 2007). This process alters the mitochondrial membrane potential and regulates release of AIF and other apoptotic mediators from the mitochondria (Elmore, 2007). The extrinsic pathway involves transmembrane death receptors such as Fas and tumor necrosis factor (TNF) receptor 1. These receptors are activated by specific ligands, such as the Fas ligand, TNF-α and TRAIL (de Jong et al., 2001; Shamimi-Noori et al., 2008). Both the extrinsic and intrinsic pathways lead to the same end, which is the execution of apoptosis by the cleavage of caspase-3, which results in DNA fragmentation (Cohen, 1997; Hirata et al., 1998). The detection of caspase-3 could therefore be a valuable and specific tool for identifying apoptotic cells in tissue sections, even before all the morphological features of apoptosis occur (Cohen, 1997; Duan et al., 2003). The closing confirmation of the apoptotic process by caspase-3 is the TUNEL method, which marks OH groups at the end of DNA fragments. Not all TUNEL-positive cells are caspase-3-positive, suggesting the existence of a caspase-3-independent pathway as well (Grant et al., 2008). Caspase-3 dependent apoptosis occurs normally during development and aging as a homeostatic mechanism which maintains cell populations in tissues (Elmore, 2007), but also represent a major mechanism by which cancer cells are eliminated (Elmore, 2007). Recent studies suggest that the flavoprotein AIF is a caspase-3 independent death effector that initiates apoptotic changes when translocated from mitochondria to

Abbreviations: Ose, ovarian surface epithelium; os, ovarian stroma; SBT, serous borderline tumor; OSC, ovarian serous carcinoma.

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the cell nucleus (Ott and Herrmann, 2009; Natarajan and Becker, 2012).

The nuclear translocation of AIF during apoptosis has been demonstrated in response to numerous stimuli and in diverse cell types (Fonfria et al., 2002; Zhang et al., 2002; Cao et al., 2003; Jambrina et al., 2003; Kim et al., 2003; Murahashi et al., 2003; Zhu et al., 2003). During embryogenesis, disruption of AIF gene was shown to inhibit the first wave of programmed cell death indispensable for mouse morphogenesis (Joza et al., 2001). During the early human gonad development, some migrating primordial germ cells expressed exclusively AIF, indicating its role in caspase-3 independent cell death (Vukusic Pusic et al., 2013). Therefore, AIF is a highly conserved protein which obeys the rules of standard evolution and represents the oldest tool to die (Aravind and Koonin, 2002; Broker et al., 2005).

In developing human ovaries, caspase-3 positivity characterizes the developing primordial germ cells, cells of surface epithelium and primary sex cords in the 5th and 6th developmental week (Vukusic Pusic et al., 2013). Recent studies indicate that during early human development both pathways of cell death operate in developing human gonads, depending on the ovarian cell type and developmental period analyzed. Furthermore, early developmental events, characterized by proliferation and subsequent differentiation of stemness cells, might be repeated and mimicked during processes of dedifferentiation in invasive tumor cells (Petricevic et al., 2011, 2012). Oct-4 (Octamer-binding transcription factor 4) a human protein encoded by gene POU5F1, is a central regulator of pluripotency (Nichols et al., 1998) and can be used as stemness marker (Samardzija et al., 2012). Expression of Oct-4 is mostly associated with the undifferentiated cell phenotype in both the developing normal tissues and tumors (Looijenga et al., 2003). It also plays an important role in renewing embryonic stem cells and their pluripotency, and is therefore often used as a marker of immature and tumor cells (Kellner and Kikyo, 2010). Disruption of Oct-4 expression can cause disturbances in the process of cell differentiation (Hochedlinger et al., 2005). So far, Oct-4 has been investigated in experimental animals, and has been proved to be involved in differentiation and in tumor formation (Samardzija et al., 2012).

The ovarian surface epithelium (OSE) represents only a small fraction of different cell types in ovaries. Their transformation to ovarian cancer cells has been the focus of investigations for the last few decades (Acquati et al., 2011). Epithelial ovarian cancers accounts for more than 90% of all ovarian tumors and still remain the most frequent cause of death. During ovarian carcinogenesis OSE have the ability to acquire Müllerian phenotype and develop into serous, endometrioid and mucinous tumors since both OSE and Müllerian tube share a common embryonic origin (celomic epithelium) (Li et al., 2012). Each of those ovarian cancers has different ranges of histological features, from the well differentiated to the undifferentiated. Among them, serous borderline (SBTs) ovarian epithelial tumors have a low potential for growth (Li et al., 2012) and are rarely associated with invasive serous carcinoma. They are considered a distinct entity unrelated to invasive carcinoma, but in some cases they progress to carcinoma, suggesting that some borderline tumors might be precursors of invasive carcinoma (Li et al., 2012).

It is possible that caspase-3 dependent apoptosis may not be the only mechanism of cell death present in ovarian carcinoma cells, and that it can be by-passed with AIF-mediated, caspase-3 independent apoptosis, which could explain the chemoresistance of the tumor cells (Lorenzo and Susin, 2007). Namely, the main malignant potential of the dedifferentiated or stemness cancer cells can be executed through avoidance of the caspase-3 dependent pathway, which has been so far accepted as a pivotal therapeutic target (Brown and Wilson, 2003; Brown and Attardi, 2005).

Previous studies have not compared the close relationship between developmental plasticity in human embryonic ovaries and OSC. In fact, it is likely that the presence of immature stem cells and caspase-3 independent apoptosis in individual tumor cells of the ovarian epithelium actually mimics the same apoptotic pathways present during normal embryonic ovary development. Therefore, in this investigation we examined these assumptions using markers of cell survival and cell death, and markers of immature stem cells during embryonic development of the human ovary and malignant transformation of ovarian epithelium.

By analyzing the expression of caspase-3 and AIF markers in the earliest developmental period of ovarian formation and in tumor cells of ovarian carcinomas, we wanted to clarify the possible role of caspase-3 independent, AIF-mediated apoptotic pathway in ovarian carcinogenesis. Confirmation of the presence of the caspase-3 independent cell death pathway in ovarian carcinomas could serve as an initial idea for development of novel therapeutic targets and pathohistological diagnostic approaches to ovarian cancers.

Materials and methods

Human material

Six normal human embryos after spontaneous abortions (5th and 8th developmental weeks) (Table 1), tumor samples of 10 ovarian serous borderline tumors (Table 2) and 10 tumor samples of 10 ovarian serous carcinomas (Table 3) (primary surgery material prior to chemotherapy) were obtained and collected from the Department of Pathology, Cytology and Forensic Medicine, University Hospital in Split, Croatia. All human embryos appeared morphologically normal; lacking signs of abnormality or macerations and a routine hematoxylin–eosin (H&E) staining was performed to verify the normal tissue preservation. The study was approved by the Ethical and Drug Committee of the School

Table 1
The human concepti analyzed in this study.

Age (weeks)	CRL (mm)	Carnegie stage	No.
5	8	14	3
8	27	22	3

Table 2
Patient characteristics for ovarian serous borderline samples.

Case	Age	Histology type
1	37	Serous borderline tumor
2	44	Serous borderline tumor
3	57	Serous borderline tumor
4	55	Serous cystadenoma papillare-borderline
5	61	Serous cystadenoma papillare
6	26	Serous borderline papillare-borderline
7	68	Serous cystadenoma papillare-borderline
8	42	Serous cystadenoma papillare-borderline, atypicum proliferans
9	44	Serous cystadenoma papillare-borderline, atypicum proliferans
10	63	Serous cystadenoma papillare-borderline, atypicum proliferans

Table 3
Patient characteristics for ovarian serous carcinoma.

Case	Age	Histology type
1	55	Adenocarcinoma papillare
2	49	Serous adenocarcinoma
3	50	Serous adenocarcinoma
4	51	Carcinoma papillare serosum
5	47	Carcinoma papillare serosum
6	79	Serous adenocarcinoma papillare
7	45	Serous adenocarcinoma papillare
8	74	Serous cystadenocarcinoma papillare
9	74	Serous adenocarcinoma papillare
10	48	Serous cystadenocarcinoma papillare