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Expression pattern of RAGE and IGF-1 in the human fetal ovary and ovarian serous carcinoma

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ABSTRACT

The expression pattern of RAGE and IGF-1 proteins in different ovarian cell lineages was histologically analyzed in six fetal, nine adult human ovaries, and nine serous ovarian carcinomas (OSC) using immunohistochemical methods. Mild expression of IGF-1 in ovarian surface epithelium (Ose) and oocytes in the 15-week human ovaries increased to moderate or strong in the stromal cells, oocytes and follicular cells in week 22. Occasional mild RAGE expression was observed in Ose during week 15, while strong expression characterized primordial follicles in week 22. In the reproductive human ovary, IGF-1 was mildly to moderately expressed in all ovarian cell lineages except in theca cells of the tertiary follicle where IGF-1 was negative. RAGE was strongly positive in the granulosa cells and some theca cells of the tertiary follicle, while negative to mildly positive in all cells of the secondary follicle. In the postmenopausal human ovary IGF-1 and RAGE were mildly expressed in Ose and stroma. In OSC, cells were strongly positive to IGF-1 and RAGE, except for some negative stromal cells. Different levels of IGF-1 and RAGE co-expression characterized fetal ovarian cells during development. In reproductive ovaries, IGF-1 and RAGE were co-localized in the granulosa and theca interna cells of tertiary follicles, while in postmenopausal ovaries and OSC, IGF-1 and RAGE were co-localized in Ose and OSC cells respectively. Our results indicate that intracellular levels of IGF-1 and RAGE protein might regulate the final destiny of the ovarian cell populations prior and during folliculogenesis, possibly controlling the metastatic potential of OSC as well.

Introduction

Ovarian cancers represent a great challenge for scientists because their etiology and pathogenesis is still poorly understood despite the existence of numerous studies. The most common histological type of epithelial ovarian cancer is high grade ovarian serous carcinoma (OSC) which is also a common lethal gynecological malignancy among women (Jemal et al., 2010). In order to understand better the histogenesis of the epithelial ovarian cancers, it is important to emphasize that during embryonic development the ovarian surface epithelium (Ose) develops from pluripotent cells of the celomic epithelium, and has the same origin as Müllerian ducts that develop into Fallopian tubes, endometrium and endocervix in adults. During ovarian carcinogenesis, Ose has the ability to acquire Müllerian cell phenotype and develop into serous (Fallopian tube), endometrioid (endometrium) and mucinous (endocervix) cancer (Shih Ie and Kurman, 2004). The high grade OSC is thought to arise *de novo* from a single layer of squamous or cuboidal cells that cover the surface of the ovary, but also from epithelial inclusion cysts that develop during the period of postovulatory healing (Cramer et al., 1983; Cramer et al., 1983). In line with this is the "incessant ovulation hypothesis" that attempts to explain the increased probability of ovarian cancer appearance by repeated minor damage of Ose due to continuous ovulations (Cooke et al., 2003). On the other hand, a study of Barker et al. (2008) proposes a theory of developmental origin of cancer (Buhimschi et al., 2009; Gebhardt et al., 2006). Unlike other tumors, where tumor progression leads to de-differentiation of epithelial cells,

Abbreviations: IGF-1, insulin-like growth factor 1; Ose, ovarian surface epithelium; RAGE, the receptor for advanced glycation endproducts; OSC, serous ovarian carcinomas.

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epithelial ovarian cancer cells usually display a higher level of differention than the surface epithelial cells from which the tumor originated (Shih Ie and Kumaran, 2004). This higher scale of differentiation level of epithelial tumor cells could also be explained by the aberrant activation of embryonic pathways in ovarian cancer histogenesis or even by reactivation of embryonic stem cells (Caric et al., 2014). Compelling evidence also demonstrated that fueling inflammation in the tumor microenvironment creates a tumor-promoting milieu, which in turn favors proliferation and survival of cancer cells (Rojas et al., 2010). It was estimated that almost 25% of all cancers are somehow associated with chronic infection and inflammation (Rojas et al., 2010) as some reliable evidence exists concerning infection and inflammation effects on tumor growth in ovarian cancer pathogenesis (Liliac et al., 2012).

Recent evidence indicates that two new markers, which are involved in normal cell proliferation and differentiation, might also have an important role in cancer pathogenesis. One of them is insulin-like growth factor 1 (IGF-1) the major mediator of the effects of growth hormone. It also plays an important role in cell proliferation, differentiation and cell survival (Hilmi et al., 2008; Yakar et al., 2005), particularly in prenatal growth through its anti-apoptotic effects in various pathophysiological conditions (Le Roith and Roberts, 2003). Studies on human tissue cultures indicate that IGF-1, as a major determinant of growth, operates in the ovary during the prenatal and postnatal periods (Baker et al., 1993; Daughaday, 1989; Yakar et al., 2005). Studies on the IGF-1 knock out mice showed that they are infertile (Baker et al., 1993). It is believed that disturbances in any of the IGF signaling pathway may be responsible for the development and progression of tumors (Yakar et al., 2005). Interest in IGF-1 and its effects on carcinogenesis has increased recently because high serum concentrations of IGF-1 are associated with an increased risk of breast, prostate, colorectal, and lung cancers (Furstenberger and Senn, 2002). So far, immunohistochemical expression of IGF-1 in human fetal ovaries and OSC has not been investigated (Le Roith and Roberts, 2003; Yakar et al., 2005). Previous investigations on human fetal ovaries and tumor tissues are either scarce or explore only certain ovarian cell lines.

The receptor for advanced glycation and products RAGE is a multifunctional receptor of the immunoglobulin superfamily, that binds to various ligands and is mainly expressed on the surface of immune cells, neurons, activated endothelial and vascular smooth muscle cells, bone forming cells, and a variety of cancer cells (Logsdon et al., 2007). RAGE mediates responses to cell damage and stress conditions, activates programs responsible for acute and chronic inflammation, and is involved in a number of pathological diseases (Riehl et al., 2009; Schmidt and Stern, 2001) including progression of cancer (Lu et al., 2010 Zhang et al., 2013). RAGE is highly expressed during development, especially in the brain, but its expression level decreases in adult tissues (Rojas et al., 2010). By now, a possible role of RAGE in tissue of reproductive human ovary was demonstrated only in a study of Diamanti Kandarakis (Diamanti-Kandarakis et al., 2007). RAGE expression has been extensively investigated in many cancer types (Logsdon et al., 2007; Rojas et al., 2010), disclosing that its expression is dictated by the accumulation of damage-associated molecules. So far, immunohistochemical expression of RAGE in human fetal and adult ovaries has not been investigated, thus the possible role of RAGE in ovaries remains unknown.

Our knowledge on the molecular function of IGF-1 and RAGE during neoplastic transformation and malignant progression is limited. However, recent experimental data including *in vitro, in vivo* and clinical studies, support a direct link between RAGE and IGF-1 activation and processes of cell proliferation, survival, cancer cell migration, and invasion (Abe and Yamagishi, 2008; Le Roith and Roberts, 2003; Liliac et al., 2012; Logsdon et al., 2007). Recent studies have shaped the conceptual framework in which cancer is viewed as a disorder that is closely related to early human development (Caric et al., 2014; Naora, 2005). Our study investigates the expression pattern of IGF-1 and RAGE during the most intensive period of proliferation and folliculogenesis in the human ovary during fetal development (developmental weeks 15 and 22), in the reproductive and postmenopausal ovaries as well as in ovarian serous carcinoma. Investigations on the possible changes in IGF-1 and RAGE protein expression pattern during normal ovarian maturation and in carcinogenesis may clarify their role in the development of ovarian cancer, thus introducing them as targets for therapeutic intervention and risk assessment. As tumor development can be regarded as a deviant form of organogenesis, aberrant activation of developmental pathways in ovarian cancer histogenesis can highlight the intimate relationship between developmental plasticity and malignant transformation.

Materials and methods

Human material and tissue processing

The study was approved by the Ethical and Drug Committee of the School of Medicine University of Split (#2181-198-03-04/10-11-0074) in accordance with the Helsinki Declaration (Williams, 2008). Ovaries of six normal human fetuses (developmental weeks 15 and 22), were obtained after spontaneous abortions from the archives of Department of Anatomy, Histology and Embryology. All tissues were morphologically normal, without signs of macerations and every tenth section was stained with Hematoxylin and Eosin to establish tissue preservation. The crown-rump length was used to determine the corresponding age of fetuses (O'Rahilly and Gardner, 1971) and correlated with the menstrual data. Five reproductive and four postmenopausal ovaries from healthy women (aged 45–53 and 56–63, respectively, who underwent oophorectomy and total abdominal hysterectomy for benign conditions), and nine high grade serous ovarian cancers (material after first surgery prior to chemotherapy) were archival materials from the Department of Pathology, Cytology and Forensic Medicine, University Hospital in Split, Split, Croatia. Histological grading of the tumors was performed according to the classification of the World Health Organization (WHO) (Ellerman et al., 2007). Tissue samples were paraffin embedded and processed as we described previously (Bartling et al., 2006; Bucciarelli et al., 2002; Poljicanin et al., 2013).

Immunohistochemical staining

Immunohistochemistry was performed as described in previous studies (Sparvero et al., 2009; Zhang et al., 2003) with some modifications. After deparaffinization and rehydration, endogenous peroxidase activity was prevented by incubation with 3% H_2O_2 for 15 min. Non-specific binding sites were blocked using 1% normal goat serum (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and diluted in phosphate-buffered saline. Sections were submitted to a water bath antigen retrieval step, immersed in Dako Target Retrieval Solution (S2367 Dako Cytomatiom, Carpinteria, CA, USA) in a microwave oven at 97 °C for 15 min. After cooling to room temperature slides were washed with PBS and then incubated with primary antibody for 1 h: goat anti-human IGF-1 antibody (AF-291-NA; diluted at 1:100, R&D Systems, Minneapolis, MN, USA) and rabbit anti-RAGE antibody (abcam3611; diluted at 1:500, Abcam, Cambridge, UK). Primary antibody binding was followed by incubation with biotinylated swine-anti-mouse, rabbit, goat antibody and with streptavidin–biotin peroxidase conjugate (K0690; LSAB+ System-HRP Dako Cytomation), both for 30 min. Antibody complexes were developed after the addition of a buffered DAB

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