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Ligand-independent androgen receptors promote ovarian teratocarcinoma cell growth by stimulating self-renewal of cancer stem/progenitor cells

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Abstract **Background**: Ovarian teratocarcinoma (OVTC) arises from germ cells and contains a high percentage of cancer stem/progenitor cells (CSPCs), which promote cancer development through their ability to self-renew. Androgen and androgen receptor (androgen/AR) signaling has been reported to participate in cancer stemness in some types of cancer; however, this phenomenon has never been studied in OVTC.
Methods: Ovarian teratocarcinoma cell line PA1 was manipulated to overexpress or knockdown AR by lentiviral deliver system. After analyzing of AR expression in PA1 cells, cell growth assay was assessed at every given time point. In order to determine ligand effect on AR actions, luciferase assay was performed to evaluate endogenous and exogenous AR function in PA1 cells. CD133 stem cell marker antibody was used to identify CSPCs in PA1 cells, and AR expression level in enriched CSPCs was determined. To assess AR effects on CD133+ population progression, stem cell functional assays (side population, sphere formation assay, CD133 expression) were used to analyze role of AR in PA1 CSPCs. In tissue specimen, immunohistochemistry staining was used to carry out AR and CD133 staining in normal and tumor tissue.
Results: We examined androgen/AR signaling in OVTC PA1 cells, a CSPCs-rich cell line, and found that AR, but not androgen, promoted cell growth. We also examined the effects of AR on CSPCs characteristics and found that AR expression was more abundant in CD133+ cells, a well-defined ovarian cancer stem/progenitor marker, than in CD133− populations. Moreover, results of the sphere formation assay revealed that AR expression was required to maintain CSPCs populations. Interestingly,

Abbreviations: CSPCs, cancer stem/progenitor cells; OVTC, ovarian teratocarcinoma; AR, androgen receptor; DHT, 5α-dihydrotestosterone; DMEM, Dulbecco's modified Eagle medium; Ct, threshold value; hrEGF, human recombinant epidermal growth factor; hrbFGF, human recombinant basic fibroblast growth factor.

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this AR-governed self-renewal capacity of CSPCs was only observed in CD133+ cells. In addition, we found that AR-mediated CSPCs enrichment was accompanied by down-regulation of p53 and p16. Finally, co-expression of AR and CD133 was more abundant in OVTC lesions than in normal ovarian tissue.

Conclusion The results of this study suggest that AR itself might play a ligand-independent role in the development of OVTC.

Introduction

Ovarian tumors are classified according to the assumed cell type of origin, namely surface epithelium (ovarian carcinoma), stroma (ovarian adenoma), salpin (fallopian tube cancer), and germ cells (ovarian teratoma) (Zhang et al., 2008; Koshy et al., 2005; Wasim et al., 2009). Ovarian teratoma, a mixed germ cell tumor, comprises approximately 20% of ovarian neoplasms (Sviracevic et al., 2011; Oliveira et al., 2004; Moniaga and Randall, 2011). Teratocarcinoma (OVTC), a very aggressive ovarian tumor, accounts for approximately 10–15% of germ cell tumor (Koshy et al., 2005; Sviracevic et al., 2011; Oliveira et al., 2004; Moniaga and Randall, 2011); however, the pathogenesis of this type of tumor is poorly understood. It is well known that sex steroid hormones such as androgens, estrogens, and progesterone play pivotal roles in reproductive function throughout reproductive ages (Walters et al., 2008; Ahmad and Kumar, 2011); however, little is known about their impact on OVTC development and progression.

Cancer stem/progenitor cells (CSPCs) are thought of as a confounding factor for carcinogenesis and cancer progression because of their capacity for unlimited self-renewal. Studies have shown that several glycoproteins, namely CD133 (Baba et al., 2009; Chen et al., 2014), CD117 (Luo et al., 2011), CD24 (Gao et al., 2010), and CD44 (Meng et al., 2012), as well as the transcription factors OCT-4 (Jiao et al., 2013) and Nanog (Lee et al., 2012), are markers of CSPCs in ovarian tissue. PA1, an OVTC cell line, is an excellent cell line for studying cancer stem cell characteristics because it abundantly expresses the CSPCs marker CD133 (Skubitz et al., 2013; Guo et al., 2011; Chung et al., 2013). In addition to using biomarkers to distinguish CSPCs, analysis of sphere-forming capacity is also a reliable method for detecting and characterizing CSPCs populations (Chung et al., 2013; Williams et al., 2010; Szotek et al., 2006; Fong and Kakar, 2010).

Androgens play important roles in both male and female reproductive organs (Chang et al., 2014; Fauser et al., 2011; Matsumoto et al., 2008). The androgens act through the androgen receptor (AR), a transcription factor that belongs to the nuclear receptor superfamily (Ma et al., 2014). AR exerts physiological and pathological functions in organisms by translocating to the nucleus upon binding to androgens, where it binds to specific DNA sequences (Wang et al., 2005, 2009; Ma et al., 2012a). However, non-classical androgen/AR action has been documented in a variety of pathophysiological conditions (Simoncini and Genazzani, 2003; Baron et al., 2004; Singh et al., 2006; Bonaccorsi et al., 2006). There are two types of non-classical androgen/AR actions: ligand-mediated transient androgen/AR actions and ligand-independent AR actions (Fujimoto et al., 2001; Heinlein and Chang, 2002; Kousteni et al., 2001; Losel et al., 2003). Ligand-mediated transient androgen/AR actions are stimulated by its binding to androgen, while ligand-independent AR actions are mediated by growth factors (Culig et al., 1994), or protein kinases (Lyons et al., 2008; Culig, 2004). However, both types of non-classical actions are the result of the translocation of AR to the nucleus where it acts as a DNA-binding transcription factor that regulates target gene expression. In this study, we examined whether androgen/AR signaling is involved in the development of OVTC in vitro and in humans.

Materials and methods

Ovarian teratocarcinoma patient data from a single cohort study

Specimens of ovarian teratocarcinoma (n = 7) analyzed in this study were obtained from patients who had received the diagnosis during the period 1987–2013 at the China Medical University (Taichung, Taiwan). Patients were identified from a single cohort registered in the Cancer Registry Database of the hospital. Access to the tissue samples was approved by the Internal Review Board of the China Medical University Hospital (#DMR101-IRB2-276).

Cell culture

The human OVTC cell line (PA1), the human embryonic kidney cell line (HEK293T), and the human prostate adenocarcinoma cell line (LnCap) were cultured in Dulbecco's modified Eagle medium (DMEM) (GIBCO, CA, USA) with 10% fetal calf serum (FCS) (GIBCO, CA, USA) and 1% penicillin/streptomycin (GIBCO, CA, USA). PA1 and LnCap cells were provided courtesy of Dr. Min-Chie Hung (MD Anderson, TX, USA) and HEK293T cells were obtained from Dr. Yuh-Pyng Shyr (Center of Molecular Medicine, China Medical University Hospital, Taichung, Taiwan). The cell lines were maintained at 37 °C in a humidified atmosphere of 5% CO_2.

Western blotting assay

Protein extraction and the immunoblot assay were performed as previously described (Ma et al., 2012b). Briefly, cells were washed with 1×PBS and resolved in RIPA buffer (100 mM Tris, 5 mM EDTA, 5% NP40; pH 8.0) with protease inhibitors (1 mM phenyl-methyl sulphonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml leupeptin). Proteins were resolved by SDS–PAGE and then transferred to PVDF membranes. Blocking of non-specific binding was accomplished by adding 5% non-fat milk. After application of primary antibodies (AR, N-20 Santa Cruz, CA, USA; β-actin, Santa Cruz, CA, USA; p53 (FL-393), Santa Cruz,