Role of cancer stem cells and microRNA in resistance to chemotherapy in patients with ovarian cancer

A. Markowska¹, S. Sajdak²

¹Department of Perinatology and Gynecology, Poznan University of Medical Sciences, Poznan
²Department of Gynecological Surgery, Poznan University of Medical Sciences, Poznan (Poland)

Summary

Despite the introduction of “the golden standard” in chemotherapy for ovarian cancer (taxanes/platinium), a relapse of the disease is noted in 80% of women treated in this manner. Studies on ovarian cancer stem cells (CSCs) and attempts at treatment using salinomycin, isolated from Streptomyces albus and endotoxin of Clostridium perfringens, are promising, in particular because CSC markers have been identified. Resistance of ovarian cancer cells to paclitaxel and cisplatin is associated with a reduced expression of miR-30c, miR-130, and miR335, which results in activation of M-CSF, the known factor of resistance to cytostatic drugs. In clear cell ovarian cancer, a reduced expression of miR-449 was detected, which may lead to overexpression of MET phenotype, typical for chemoresistant ovarian cancer. MicroRNAs remain in investigations, but their involvement in the control of genes linked to the development of the cancer and its progression seems to offer the promise of a targeted therapy.

Key words: Ovarian cancer; Cancer stem cells; MicroRNA; Resistance to chemotherapy.

Introduction

Chemotherapy aims at injuring genetic material or damaging the structures of a neoplastic cell significant for growth and division, leading to apoptosis of the cell and its elimination. The applied cytostatic drugs are toxic and activate several defensive mechanisms such as pumping the drug out of the cell, detoxification in the cell, mobilization of DNA injury detection, and its repair. A lowered or absent sensitivity to cytostatic drugs is linked to multiple molecular mechanisms, including multidrug resistance (MDR) depending on MDR genes and inhibition of apoptosis, conditioned by several genes. The mechanisms have been studied for several years and are relatively well-recognized. Over the last decade investigators have focused on other causes of chemotherapy failure: cancer stem cells and microRNA.

Cancer stem cells (CSCs)

At the start of chemotherapy, most ovarian cancer cells are sensitive to such treatment, resulting in an effective reduction in tumour weight. However, in approximately 80% of patients this is followed by a relapse of the disease which during exposure to consecutive cytostatic drugs develops an acquired resistance and finally leads to an unfavourable outcome. The currently accepted hypothesis states that the principal cause of cancer relapse, chemoresistance and metastases involves cancer stem cells (CMCs), also known as cancer initiating cells (CICs). They involve a small cell subpopulation within a frequently heterogenous ovarian tumour, generally comprising less than 2% of its weight and already present in the tumour prior to chemotherapy [1-3].

The chemoresistance of CSCs is most probably linked to their properties [3-6]: capacity for self-renewal and differentiation, their resting state (low mitotic index), high levels of active trans-membranous ABC transporters (ATP-binding cassette family), including P glycoprotein (P-gp), the multidrug resistance associated protein (MRP1), and the breast cancer resistance associated protein (BCRP). The drug can be therefore be actively eliminated from the neoplastic cells (the drug is "pumped out") through activity of apoptosis-inhibiting pathways and capacity to DNA repair.

The key signalling pathways involved in the survival of CSCs and the related resistance involve the following conservative pathways: Wnt, controlling growth of CSC and equipping them with the ability of self-renewal; notch, significant for their survival, proliferation, and intercellular interactions; and hedgehog, also engaged in proliferation and differentiation of CSC [3, 6-8]. Cancer stem cells are identified and isolated by their markers; CD44, CD117, CD133, Bmi1, Nestin, Oct-4 (POUSF1), and Nanog [9-11] are considered typical for ovarian cancer.

CD44+ and CD117+ cells are known also as ovarian cancer initiating cells (OCIC); they manifest pronounced proliferation and invasiveness. Their activity develops with the...
mediation of the stromal derived factor (SDF1) cytokine and its receptor CXCR4 [12]. Moreover, CD44+ cells may bind to Nanog, the nuclear transcription factor, and can activate STAT3, in this way affecting the expression of multidrug transporters, including MDR1 (P-gp), inducing the elimination of a drug from the cells. A similar effect results from binding CD44+ with hyaluronic acid (HA), the principal component of the extracellular matrix. The HA-CD44+ interaction indirectly induces P-gp, with the resulting chemoresistance [13]. Additionally, overexpression of the trans-membranous glycoprotein CD133 (promine) is linked to unfavourable prognosis in ovarian cancer due to its involvement in chemoresistance, similar to the expression of the nuclear transcription factors: Bmi1, Nanog, and Oct-4.

Overexpression of another stem cell marker, Nestin, a type VI filament protein, also correlates with poor prognosis in ovarian cancer [14]. Studies on human ovarian cancer xenografts in mice as well as in vitro and in vivo experiments demonstrated that expression of chemoresistance to cytostatic drugs (paclitaxel, carboplatin, cisplatin) is followed by an increase in the number of cells with CSC markers, which indicates that the cells are insensitive to cytostatic drugs [10, 15]. Zeng et al. [9] examined the expression of six CSC markers in women with primarily diagnosed or relapsing ovarian cancer. Expression of CD133, CD117, and Bmi1 was markedly elevated in relapsing tumours as compared to primary tumours; no differences could be demonstrated in the expression of CD44, Nestin, and Oct-4. In the opinion of the authors, the heterogeneity of ovarian CSCs deserves further studies.

Studies continue on the identification of CSC markers, as in the Wnt, Notch or Hedgehog signalling pathways, they are likely to be applied in targeted therapy [3, 12, 16]. Trials of an anti-CSC therapy are underway using salinomycin, the antibiotic isolated from Streptomyces albus, capable of inducing activation of caspase 8, the executive enzyme of apoptosis in ovarian cancer cells [17]. Promising results were detected in an animal model using endotoxin of Clostridium perfringens [18].

**MicroRNA**

MicroRNA represent a family of small, 19-22 nucleotide, non-coding RNA molecules, involved in controlling overexpression of other genes, at the stage of translation of genetic information. MicroRNA may control both the expression of oncogenes and tumour suppressor genes. Their effect on tumour development, through promotion of proliferation, invasiveness, migration and angiogenesis, and by reduction of apoptosis, may reflect whether an increased expression of microRNA or its reduction, depending on the molecular target involved. If microRNA genes are located in sites undergoing amplification, and the effect involves a reduced expression of a suppressor gene, they exert an oncogenic influence. Similarly, if microRNA genes are located in regions undergoing a deletion and targets of their control involve oncogenes, they also exert an oncogenic influence. MicroRNAs were also demonstrated to exert an inhibitory effect on the development of a tumour, due to an elevated expression of a tumour suppressor and oncogen expression silencing [19-22].

Numerous investigations detected links between microRNAs and chemoresistance; their involvement in MDR mainly involves the control of MDR proteins at the post-translation level [1, 23-25]. Sorentino et al. [24] analysed the expression of six selected microRNAs in cell lines of ovarian cancer resistant to paclitaxel and cisplatin. Three of them, including miR-30C, miR-130a, and miR-335, demonstrated decreased expression in all the cell lines resistant to the cytostatic drugs studied. The expression of miR-130a was shown to be linked to the activation of translation involving the M-CSF gene, a known factor of chemoresistance in ovarian cancer. Jang et al. [25] also examined the expression of six microRNAs in ovarian clear cell carcinoma and in serous carcinoma of the ovary, as well as in endometroid carcinoma and clear cell carcinoma of the endometrium. This unique study was conducted in Korea, where clear cell carcinomas manifest high incidence and the cancers manifest resistance to treatment with taxanes and platinum, linked to poor prognosis [26]. Jang et al. [25] detected low expression of miR-449 as a common trait of “gynaecological” clear cell carcinomas. Mir-449 represents a tumour suppressor, targeted at MET; low expression of MIR-449 leads to overexpression of MET and to the phenotype of chemotherapy-resistant clear cell carcinoma.

High grade serous ovarian cancer (HGSOC) represents an aggressive type of ovarian cancer which poorly responds to chemotherapy. According to Gorzel et al. [27], a high expression of miR-433 in studies on ovarian cancer cell lines is associated with development of chemoresistance. The distinct influence of microRNA expression on chemoresistance was described by Li et al. [22] in their studies on xenometastases of ovarian cancer cell lines in mice. In ovarian cancer cell lines resistant to cisplatin, the expression of miR-128 was reduced. However, administration of cisplatin with agonists of agonist of miR-128 was followed by an effective reduction in tumour weight while expression of ABCC5 and Bmi-1 proteins, linked to resistance to cisplatin, were lower. The authors expressed an opinion that such a procedure administering agonists of certain microRNAs offers promising therapeutic aspects.

Zong et al. [23] also examined the effect of microRNA on chemoresistance. In their studies, cell lines of ovarian cancer following transfection with mi-R130b demonstrated a lowered sensitivity to paclitaxel and cisplatin as compared to the control group. Therefore, miRNA-130b may also be linked to the development of chemoresistance of ovarian cancer to cytostatic drugs.

Interesting experiments associated with the activity of microRNA - MIR152 were conducted by He et al. [28]: in tests on cell lines of ovarian cancer they demonstrated that autophagy (autophagocytosis) plays an important role in resist-
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ance to cisplatin, representing an alternative pathway to apoptosis and involving the controlled decay of proteins with an extended half-life and the removal of cell organelles, indispensable in the acquisition of energy sources and for cell survival. While induced in response to chemotherapy, it also provides a mechanism of chemoresistance development due to the removal of damaged organelles and cytoprotection [29]. In cultures of ovarian cancer cells resistant to cisplatin, low expression of MIR152 was demonstrated, while overexpression of MIR152 reduced autophagy induced by cisplatin, sensitized ovarian cancer cells to cisplatin and induced apoptosis.

Another mechanism of resistance to chemotherapy involves the interaction of microRNA with anoikis (from the Greek word for homeless), a type of programmed cell death induced by the broken interaction of epithelial cells with the matrix [1, 30]. Resistance against anoikis allows tumour cells to survive, migrate, and metastasize despite their lack of contact with the matrix; it is linked to resistance to chemotherapy [1]. In studies by Citelly et al. [31] both ovarian cancer cell lines and cancer cells in the third stage of advancement were found to manifest low expression of mir-200C, which was linked to poor prognosis. Recovery of miR-200C in xenografts of ovarian cancer cells in the third stage of advancement were found to manifest low expression of mir-200C, which was linked to poor prognosis. Recovery of miR-200C in xenografts of human ovarian cancer influences their chemosensitivity to cisplatin, sensitized ovarian cancer cells to cisplatin-induced apoptosis by inhibiting cyto-protective anoikis.

References


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