Review

Mechanisms of apoptosis in ovarian cancer: The small molecule targeting

Philemon, N. Ubanako, Mpho Choene and Lesetja Motadi*

School of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg, South Africa.

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Ovarian cancer is the most lethal gynaecologic cancer cancer among women. About 90% of ovarian cancers are epithelial, (ovarian carcinomas) thought to arise from the ovarian surface epithelium. Diagnosed usually at clinically advanced stages, many patients show poor response to chemotherapy, with resistance and recurrent disease being prevalent. The cell origin and the mechanism of neoplastic transformation of this malignancy are poorly understood. Apoptosis is crucial in normal ovarian development and function; and gonadotropins play a significant role in modulating the expression of several pro-apoptotic and pro-survival genes and other molecules in the ovary. Targeted therapeutic strategies using small molecule protein kinase inhibitors and monoclonal antibodies have been explored in the management of ovarian carcinomas. These molecules, used in combination with chemotherapy, have shown better prognosis in ovarian cancer. With several ongoing clinical trials using kinase inhibitors and the ideal targets being sought after, significant improvements of patients suffering with ovarian carcinomas are expected in the near future. This manuscript aims to review ovarian apoptotic mechanisms and the therapeutic progress in the use of small molecule kinase inhibitors and monoclonal antibodies as targets for inducing apoptosis in ovarian cancer.

Key words: Ovary, apoptosis, gonadotropins, targeted therapy.

INTRODUCTION

Ovarian cancer is the leading cause of death among all gynecological cancers in western countries. When compared to other gynecological malignancies, the mortality rate of ovarian cancer exceeds that of cervical and uterine cancers put together (Siegel and Jemal, 2013). This high death rate is due to the diagnosis at an advanced stage in most patients which is caused by the relative lack of specific signs and symptoms of the disease, and the lack of reliable tests for early detection. Disease-associated mortality in ovarian cancer is as a result of metastasis, which is characterized by ascites, peritoneal and systemic dissemination.

Despite platinum and paclitaxel-based chemotherapy plus cyto-reductive surgery in ovarian cancer, less than one-third of patients will survive after 5 years (Cannistra, 2004; Zhang et al., 2009; Gavalas et al., 2010; Kim et al., 2012). There are more than 30 types and subtypes of ovarian cancer; however, they have been classified into 3 major categories according to the primary cells from which they arise. They are categorized as either: epithelial tumours, stromal tumours or germ cell tumours (Kaku et al., 2003). Epithelial ovarian tumours (ovarian
Apoptosis as cell division guardian

Apoptosis is a form of cell death in which a programmed sequence of events leads to the elimination of cells without releasing harmful substances into the surrounding area (Kerr et al., 1972). Apoptosis plays a crucial role in developing and maintaining the health of the body by eliminating old, unnecessary and unhealthy cells. Two main pathways of apoptosis have been elucidated: the death receptor (extrinsic) pathway and the mitochondrial (intrinsic) pathway of apoptosis. The extrinsic pathway of apoptosis involves binding of ligands to cell surface receptors of the tumour necrosis factor-alpha (TNF-α) superfamily. The death receptor-ligand binding leads to transmission of the death signal through death domains, death effector domains and caspases recruitment domains. The caspase recruitment domains cause the activation of procaspases and adaptor proteins. Procaspases are then cleaved to form active caspases which lead to apoptosis (Hussein, 2005). The intrinsic pathway is regulated by members of the Bcl-2 family. Upon reception of a death signal such as ultra violet rays, gamma radiation oncogenic activation or chemotherapeutic agents by the cell, pro-apoptotic members of the Bcl-2 family are stimulated. This leads to formation of pores on the mitochondrial membrane enabling the release of cytochrome complex out of mitochondria into the cytoplasm (Hussein et al., 2003). Cytochrome c then binds to Apaf 1, alongside dATP to form the apoptosome complex (Gewies, 2003; Spierings et al., 2005). The apoptosome complex cleaves caspase 9, which in turn activates a cascade of events involving executioner caspases resulting in apoptosis (Gupta, 2001; Spierings et al., 2005).

Role of apoptosis in ovarian follicle development and maintenance

The ovary provides a paradigm for programmed cell death due to the cyclic nature of ovarian development and function (Hussein, 2005). Apoptosis is critical for ovarian function, bearing in mind that it regulates the cyclical processes in the female reproductive system. Apoptosis has been shown to be the underlying mechanism of cell death in the ovaries. It has been observed in germ cell loss (germ cell attrition), follicular atresia, corpus luteum regression (luteolysis) and ovarian surface epithelial cells prior to ovulation (Murdoch 1995, Murdoch and McDonnel, 2002; Tilly, 1996; Hussein, 2005).

Apoptosis in germ cell attrition

About seven million oocytes are produced in the ovary during the early life of the human foetus. However, there is a drastic reduction of the number of oocytes to about one third by apoptosis, shortly after birth. The reasons for such a dramatic depletion of the follicular reserve are not yet known. However, Monniaux (2002) proposed that quality control (for the elimination of genetic defects) and a deficit of survival factors from adjacent cells are possible mechanisms. Mutant mice, gene-knock out or transgenic mice have confirmed the involvement of certain molecular effectors in germ cell attrition. Perez et al., (1999), showed that Bax knock-out mice, though normal at birth, increased the number of oocytes during puberty and during their fertile lives. Bcl-2 (B-cell lymphoma
2) transgene mice showed increased folliculogenesis and development of teratomas (Hsu et al., 1996), and knockout experiments also showed loss of oocytes (Ratts et al., 1995). Defects in apoptosis or sustained proliferation of germ cells may lead to germ cell tumours of the ovary.

Follicular atresia

Follicular atresia is the breakdown and resorption of ovarian follicles which occurs prior to ovulation (Santos et al., 2008). Several studies have shown that apoptosis of granulosa cells is the main mode of cell death in follicular atresia (Tilly et al., 1991; Kaipia and Hsueh, 1997; Manabe et al., 2004). Surging Follicle-stimulating hormone (FSH) levels during follicle recruitment enables some antral follicles to evade apoptotic death. A dominant follicle escapes death by secreting oestrogen and inhibin. FSH is thereby, inhibited by inhibin via a negative feedback mechanism. This leads to a negative selection by apoptotic death of the remaining follicles in the group (Hussein, 2005). A positive selection of the dominant follicle is simultaneously enforced by the local secretion of growth factors (McGee and Hsueh, 2000). The dominant follicle is subsequently ovulated. A handful of ligands and their receptors have been shown to be involved in the process; albeit the list of biomolecules continues to expand. They are: TNF-α, TNF-related apoptosis inducing ligand (TRAIL), Fas ligand and APO-3 ligand (Manabe et al., 2008; Kaipia et al., 1996; Quirk et al., 1995). They induce apoptosis via the extrinsic pathway, although the mechanism of TNF-α mediated apoptosis is not known. The fate of the cell is decided by a fine balance between pro-survival and pro-apoptotic molecules. Pro-survival molecules such as insulin-like growth factor-1 (IGF-1), gonadotropins, epidermal growth factor, B-cell lymphoma-extra large (Bcl-xL), Bcl-2, X-linked inhibitor of apoptosis protein (XIAP), Neuronal apoptosis inhibitory protein (NAIP), and integrins are involved in keeping the cell alive, while anti-survival molecules such as, bax, Fas antigens, p53, TNF, Fas/Fas ligand, granzyme-B, GATA-4, IGF favour cell death (Gospodarowicz et al., 1977; Driancourt et al., 1998; Besnard et al., 1996; Matsumoto et al., 1999; Vaskivuo et al., 2001; Amsterdam et al., 2003; Tilly, 2001; Jiang et al., 2003; Hussein, 2005; Caldas et al., 2006; Aoudjit and Vuori, 2012).

Corpus luteum regression (Luteolysis)

Luteolysis is the degeneration of the corpus luteum which occurs at the end of the female reproductive cycle in the absence of pregnancy (Vaskivuo and Taipanainen, 2003). Luteolysis is comprised of both structural and functional processes. The functional process is characterized by an initial drop of progesterone secretion. On the other hand, the structural process is characterized by changes in the morphology of the cells of the corpus luteum, accompanied by an involution to a smaller mass of whitish connective tissue called the corpus albicans. The corpus albicans often persists in the ovary for several weeks. Data exists to prove unequivocally that apoptosis is the mechanism of luteolysis in humans (Shikone et al., 1996; Vaskivuo et al., 2002). The molecular effectors that have been shown to be mediators of luteolysis include Fas/Fas ligand, prostaglandin F2-α, endothelins, integrins and interferon-γ (Moeljono et al., 1977; Quirk et al., 1995; Otani et al., 1996; Petroff et al., 2001; Wall et al., 2003).

Apoptosis in ovarian cancer and its therapeutic targets

We have made substantial progress in our understanding of cancer biology and genetics over the past decade. The realization that a malignant phenotype is greatly affected by apoptosis and its related genes is of paramount importance. It is now clear that tumour initiation, transformation or metastases are induced by some oncogenic mutations that disrupt apoptotic mechanisms (Lowe and Lin, 2000). Moreover, it is now well known that most cytotoxic chemotherapy induce apoptosis, suggesting that dysfunctional apoptotic mechanisms significantly contribute to treatment failure. Targeted therapy takes advantage of the fact that apoptotic pathways can be manipulated to induce significant apoptotic cell death by targeting specific genes and proteins that regulate such pathways. Recently, most anticancer agents now in use were developed with the sole purpose of inducing apoptosis that will selectively kill tumour cells. So, targeting apoptosis remains the main focus and safest route towards combating cancer.

Targeted therapy in ovarian cancer

Despite optimal chemotherapeutic and surgical treatment of ovarian cancer patients presenting with advanced disease, 10 to 15% show long-term subsidence. More than 70% of chemotherapeutically treated ovarian cancer cases show resistance, and subsequent relapse to platinum-based drugs and paclitaxel (Copeland et al., 1994; Bartel et al., 2008; Gavalias et al., 2010). This necessitates research into alternative therapeutic strategies against ovarian cancer. Conventional cancer chemotherapy is cytotoxic, and indiscriminately targets rapidly-dividing cells of the body. In a bid to destroy cancerous cells, some normal body cells are killed in the process, resulting in serious side effects (American Cancer Society, 2006). Damages to hair follicular cells, bone marrow cells, gastro-intestinal cells and cells lining the reproductive tract, account for a great proportion of
side effects experienced by chemotherapeutic use. Also, additional limitations of traditional chemotherapy include the palliative and unpredictable responses produced by patients (Arora and Scholar, 2005).

However, targeted therapy depicts a new generation of anti-cancer therapeutics that are designed to ideally interact with a specific molecule, usually a protein molecule, that is believed or shown to have a vital role in tumour progression or growth (Wu et al., 2006; Arora and Scholar, 2005). Researchers have proposed a set of criteria that qualify an ideal molecular target in cancer therapy. They include the following:

1. The molecule should be significantly expressed in vital tissues.
2. It should be reproducibly measured in clinical samples.
3. It should correlate with clinical outcome; yielding a clinical response in a significant amount of patients whose tumours expresses the target, and shows a trivial response in those whose tumours don not express the target (Ross et al., 2004).

However, side effects have been reported in numerous clinical trials involving small molecule tyrosine kinase inhibitors some of which are as a result of unintended targets. They include fatigue, bowel perforations and severe diarrhea, hypertension, hand-foot syndrome and proteinuria as observed in some vascular endothelial growth factor receptor (VEGFR) and epidermal growth factor receptor (EGFR) inhibitors (Friedlander et al., 2007; Azad et al., 2008; Nimeiri et al., 2008; Biagi et al., 2008).

As opposed to conventional chemotherapy, a hallmark of the action of targeted cancer therapeutics is a higher degree of selective toxicity. This aspect of an anticancer drug may be improved upon by either augmenting the concentration of the therapeutic agent that reaches the tumour tissue or decreasing the amount that reaches normal tissues (Singh et al., 2010). However, synergistic anticancer effects have been achieved by using targeted therapeutic approaches in combination with cytotoxic chemotherapy (Aurora and Scholar, 2005). Various targeted therapeutic strategies have been explored in ovarian cancer management, often used in combination with chemotherapeutic agents for maximal results. Targeted cancer therapeutics can be grouped into two broad categories: small molecule inhibitors and monoclonal antibodies (Wu et al., 2006; Aurora and Scholar, 2005). However, targeted therapy also encompasses RNA inhibition strategies which have been also explored in this manuscript.

**Targeting Bcl-2 Family in ovarian cancer and apoptosis**

Bcl-2 family of proteins is divided into two types of proteins: those that can induce apoptosis referred to as pro-apoptotic and those that inhibit apoptosis called anti-apoptotic molecules. The most common protein which is well defined is Bcl-2 which is an anti-apoptotic molecule that exerts its effects by binding to Bax, blocking c-Myc-induced apoptosis, blocking mitochondrial release of cytochrome-c and also inhibiting Apaf-1 interaction with Caspase-9 (Luo et al., 1997).

In the ovaries, Bcl-2 is expressed mainly in healthy ovarian follicles while Bax, a pro-apoptotic molecule, is expressed in the follicles undergoing atresia. Bcl-2 and Bax expressions are markedly influenced by gonadotrophin levels. Elevated gonadotropins tend to inhibit Bax expression while increasing Bcl-2 and Bcl-xL expression, hence promoting the survival of the follicle (Tilly et al., 1995; Sugino et al., 2000). Tilly et al. (1995), analyzing the expression of Bcl-2 family of protein in the immature ovaries of a rat during follicular atresia found the correlation between Bax mRNA which was upregulated and that of Bcl-2 and Bcl-xL down-regulated. Other pro-apoptotic molecules such as Mcl-1, Bax and Bok elicit their apoptotic effects by triggering mitochondrial cytochrome-c release. Cytochrome-c binds to Apaf-1, forming the apoptosome and activating the Caspase cascade which leads to apoptotic cell demise (Tilly et al., 1995).

Because of the importance of pro- and anti-apoptotic proteins in deciding if the cell undergoes apoptosis or they survive, they have become targets for many researchers who are eager to restore apoptosis in cancer cells. Ovarian cancer is mainly associated with mutations in some of the genes that might trigger apoptosis and restoring their functions might be alternative therapeutic options. In recent years, several small molecules that aim to target Bcl-2, thereby inhibiting its activity in cancer cells have been identified as possible therapeutic options. One of the recently discovered molecules that act as a selective inhibitor of Bcl-2 is AB-737 and its orally active product AB-263 were shown to inhibit cell growth in eight different ovarian cancer cell lines, although with relatively poor potency. Further tests revealed that ABT-737 increased the sensitivity of several cell lines to carboplatin when ABT-737 was administered after carboplatin (Witham et al., 2007; Jain et al., 2014). In addition, ABT-737 significantly increased carboplatin activity in patients recently treated with chemotherapy (Witham et al., 2007). Co-administration of carboplatin and siRNA directed against Bcl-2 or Bcl-xL resulted in a high growth inhibition in tumour xenographs as compared to carboplatin administration alone (Witham et al., 2007). In recent clinical trials, ABT-737 is able to prolong the survival of recipient mice transplanted with Bcl-2-transduced tumors. It was also found to be functional with co-treatment with chemotherapy.

The second most common molecule which aims to target Bcl-2 is AT101, the importance of this molecule unlike ABT-737 is that it can bind with high affinity to Bcl-xL, Bcl-2 and Mcl-1 and it induces cell apoptosis by activating Bax through a conformational change, trans-
location, and oligomerization that lead to cytochrome c release and effector caspase 3 cleavage (Zerp et al., 2009). Another promising molecule which is on clinical trials phase I and II is Obatoclax (GX15-070), a small-molecule inhibitor of antiapoptotic Bcl-2 proteins which has been reported to trigger cell death via autophagy in ovarian cancer cells (Basit et al., 2013). In another study in a panel of ovarian cancer cell lines, selected for oxaliplatin resistance, Crawford et al. (2011) showed that Obatoclax was able to decrease cell viability irrespective of platinum resistance status. While another molecule HA14-1 is said to inhibit Mcl-1 thereby increasing cell death in ovarian cancer cell lines. Mcl-1RNA interference assisted HA14-1 to induce apoptosis in the absence of chemotherapy (Simonin et al., 2009).

Minimizing expression of Inhibitors of Apoptosis (IAP) as targets for ovarian cancer

The inhibitor of apoptosis proteins (IAPs) constitutes a family of highly conserved group of proteins that are involved in apoptosis, immunity, inflammation cell cycle regulation and migration (Lopez and Meier, 2010). Although, IAP homologs have recently been demonstrated to suppress apoptosis in mammalian cells, their expression and role in human ovarian epithelial cancer and chemotherapy resistance are still unknown or unclear. To date, eight IAPs have been identified: NAIP (BIRC1), c-IAP1 (BIRC2), c-IAP2 (BIRC3), X-linked IAP (XIAP, BIRC4), Survivin (BIRC5), Apollon (BRUCE, BIRC6), Livin/ML-IAP (BIRC7) and IAP-like protein 2 (BIRC8) (Vucic and Fairbrother, 2007). IAPs, inhibit the caspases by binding their conserved BIR domain to the active sites of caspases. This in turn either induces the degradation of active caspases or the separation of caspases from their respective substrates (Wei et al., 2008). Dysregulated IAP expression has been reported in many cancers. Abnormal expression of IAPs, which also correlated with resistance to chemotherapy, has been shown in pancreatic cells (Lopes et al., 2007).

When designing novel drugs for cancers, the IAPs are attractive molecular targets. So far, XIAP has been reported to be the most potent inhibitor of apoptosis among all the IAPs. It effectively inhibits the intrinsic as well as extrinsic pathways of apoptosis by binding and inhibiting upstream caspase-9 and the downstream caspases-3 and -7 (Svane et al., 2004). In normal ovaries, surging FSH in the ovary upregulates XIAP which leads to a suppression of granulosa cell apoptosis and promotes the growth of follicles induced by FSH (Hussein, 2005; Xiao et al., 2001; Scott et al., 2005). So if this is true even during ovarian cancer development, XIAP might provide much better molecular therapeutic target in chemo-resistant ovarian cancer. In recent years, some novel therapy targeting XIAP included short interfering RNA (siRNA) molecules and anti-sense strategies. In using the antisense approach, Dai et al. (2009) reported that inhibition of XIAP resulted in an improved in vivo tumour control by radiotherapy. In another study by Li et al. (2001), they have shown through the use of cisplatin-sensitive and -resistant human ovarian surface epithelial (hOSE) cancer cell lines and adenoviral antisense and sense complementary DNA expression to examine the role of IAP in the regulation of apoptosis in human ovarian cancer cells and chemoresistance. Cisplatin consistently decreased XIAP content and induced apoptosis in the cisplatin-sensitive, but not cisplatin-resistant, cells.

Resistance to cisplatin in ovarian cancer, arises from the dysregulation of tumour suppressors and survivals signals. Piceatannol which is a natural metabolite of the stilbene resveratrol found in grapes and is converted from parent compound by the enzyme CyP1BA/p450. Piceatannol was found to enhance p53-mediated expression of the pro-apoptotic proteins NOXA, and also increased XIAP degradation via the ubiquitin ligases and enhance caspase-3 activation (Farrand et al., 2013). Another recently identified molecule that seemed to suppress the expression of XIAP is LBW242, which is a small molecule that mimics SMAC/DIABLO this molecule was shown to activate apoptosis in both primary ovarian cancer cells and ovarian cancer cell lines by activating caspase-8 (Eschenburg et al., 2012; Petrucci et al., 2012). LBW242 also sensitizes ovarian cancer cells to the antitumor effects of TRAIL and commonly used anticancer drugs. Birinapant (TL32711), a synthetic small molecule and peptidomimetic of second mitochondrial-derivative activator of caspases (SMAC) and inhibitor of IAP family of proteins, has demonstrated strong correlation between drug exposure, target coverage and apoptosis induction in tumours at well-tolerated doses as well as promising anti-tumour activity in patients (Nguyen et al., 2013).

Therapeutic potential of TNF family members

Tumor necrosis factor alpha (TNF-α), is the prototypic ligand of the TNF superfamily. It is a pleiotropic molecule that plays a key role in inflammation, apoptosis and development of the immune system. In normal ovarian development, the expression of some TNF family members such as FasL/Fas is highly influenced by gonadotrophin levels. Surging gonadotrophin levels result to a decreased expression of Fas/FasL, thereby promoting follicular survival. However, decreased gonadotropin levels result in an increased expression of Fas/FasL, leading to follicular atresia (Jiang et al., 2003). TNF-α is highly expressed in ovarian cancer. TNF-α modulates the expression of CD44 in normal T lymphocytes and CD44 is involved in the carcinogenesis and metastasis of ovarian cancer (Muthukumaran et al., 2006). TNF family members show both pro-survival and
pro-apoptotic functions, depending on the type of receptors that are activated. TNF-α triggers apoptosis by activating caspases (Wang et al., 2008); on the other hand, TNF-α is able to aid the survival of granulosa cells by upregulating the expression of and XIAP through the NFκB system (Jiang et al., 2003). TRAIL is another TNF family member that has been shown to induce apoptosis in tumour cells, but not in normal cells, owing to the presence of TRAIL decoy receptors which competitively inhibit the binding of TRAIL ligands to their cognate receptors (Sheridan et al., 1997; Pan et al., 1997). TRAIL and its receptors are expressed in growing, atretic and antral ovarian follicles (Bobe and Goetz, 2001).

**Wt p53: the genomic guardian target**

TP53 is a gene that encodes the tumour suppressor protein, p53, which contains a DNA-binding domain, a transcriptional activation domain and an oligomerisation domain (Harms and Chen, 2006). P53 responds to various cellular stresses to regulate the expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism (Bates and Vousden, 1999). Mutations in this gene are associated with a variety of human cancers (Toledo and Wahl, 2006). The effects of p53 are mainly as a result of its activity as a transcription factor. It transcriptionally activates pro-apoptotic genes such as Bax, PUMA, NOXA and the growth arrest gene p21. It represses the transcription of anti-apoptotic genes such as Bcl-2 and Bcl-xL, MDM2 (Barak et al., 1994; Wu et al., 2001; Ohtani et al., 2001). p53 is expressed in apoptotic granulosa cells of atretic follicles, suggesting a possible role of p53 in follicular atresia (Kim et al., 2000). One of the ways by which Gonadotropins inhibit apoptosis is by repressing a host of pro-apoptotic genes including p53 gene expression (Tilly et al., 1995). It has been shown that mutant p53 can be refolded to the wild type conformation by an active compound, MQ, which is converted from the inactive form, APR-246. This leads to p53-dependent apoptosis (von Euler et al., 2014).

In addition to reactivating p53 in ovarian cancer, APR-246 also decreases the intracellular levels of glutathione in a dose-dependent fashion (von Euler et al., 2014). The targeting of both p53 and glutathione probably accounts for the strong synergistic effects of APR-246 and platinum-based drugs in ovarian cancer cells (von Euler et al., 2014). Ad5CMV-p53 is a recombinant adenoviral vector that encodes TP53. The co-administration of Ad5CMV-p53 intraperitoneally with chemotherapy using gemcitabine, showed synergistic effects in the treatment of recurrent disease after multiple cycles of therapy (Wen et al., 2003). In another study, it was shown that Ad5CMVp53 showed a marked clinical activity when used in combination with platinum-based chemotherapy to treat recurrent ovarian carcinoma (Buller et al., 2002). Cytoplasmic-Domain-of-Band-3-Protein-(cd3), which is a synthetic p53 binding protein-derived peptide interacts with the core domain of p53, and upregulates the transactivation activity of p53 (Samuels-Lev et al., 2001). A fluorescently tagged Cytoplasmic-Domain-of-Band-3-Protein-(cd3) was shown to improve the proper native folding of p53Arg273His contact and p53Arg175His mutants; induce p53-mediated transcriptional activation of p21, GADD45 and MDM2 and induce apoptosis. Even though the wild-type bearing cells did not undergo apoptosis after treatment with Cytoplasmic-Domain-of-Band-3-Protein-(cd3) alone, the cells showed enhanced sensitivity to apoptosis induced by infra-red radiation (Issaeva et al., 2003).

A small synthetic molecule of the styrylquinazoline family known as Cp-31398 has been identified for its ability to stabilize p53 against thermal denaturation in vitro. Treatment with Cp-31398 hinders p53 ubiquitination and degradation leading to an enhanced cell surface exposure of DR5 and an activation of the intrinsic bax/mitochondrial/caspase-9 pathway, culminating in apoptosis (Vecil et al., 2003). Because of the critical inhibitory role of MDM2 and RBBP6 on p53 (Li et al., 2007), blocking the interaction of RBBP6, MDM2 and p53 has been proposed as a potential cancer therapeutic strategy (Moela et al., 2014). Nutlin-2 is a small-molecular-weight inhibitor that fits into the pocket where wild-type TP53 binds to MDM, a molecule required for the rapid degradation of TP53 through the ubiquitin-proteasome pathway (Wang et al., 2012). Inhibition of the MDM–TP53 interaction results in the increased expression of wild-type TP53, inhibiting tumor growth and inducing apoptosis. In a study by Moela et al. (2014) have shown that silencing breast cancer cell lines with RBBP6 siRNA followed by treatment with camptothecin also sensitized cancer cells to apoptosis induced cell death which serves as a promising natural cell biology pathway.

**Other apoptotic molecular targets for ovarian cancer cells**

**Interferons (IFN)**

They include IFN-α, IFN-γ, IFN-β and IFN-δ. IFN-γ is a cytokine that has potent immunomodulatory, antiviral, and antiproliferative that has anticancer activity. IFN-γ directly inhibits human tumor cell growth and induces apoptosis (Clemens, 2003). Interferons sensitize cells to apoptosis-inducing genes and proteins in apoptotic pathways (O’Connell et al., 2000; Lissat et al., 2007). IFN-δ inhibits Fas expression in the corpus luteum, thereby inducing apoptosis in bovine luteal cells (Komatsu et al., 2003). This is as a result of anti-apoptotic Fas effects in the corpus luteum. IFN-γ has been shown to induce apoptosis in luteal cells and ovarian cancer cells both in vivo and in vitro (Petroff et al., 2003).
In ovarian cancer, cell lines and xenografts in nude mice IFN was found to induce apoptosis and inhibit proliferation of cancer cells (Wall et al., 2003). IFN-γ might be a useful biological treatment of human epithelial ovarian cancer if sustained levels of this cytokine could be achieved within the peritoneum by improved protein or gene delivery strategies.

**GATA-4**

GATA-4 is a zinc finger transcription factor that induces cell proliferation. GATA-4 expression during foetal development is expressed in granulosa cells of primary and antral follicles (Orkin, 1992). GATA-4 expression has been shown to correlate with active granulosa cell proliferation in adult human ovaries; therefore possibly functioning as a pro-survival molecule in the ovary (Vaskivuo et al., 2001). In adult mouse ovaries, GATA-4 down-regulation correlates with follicular atresia. GATA-4 expression has been shown to be stimulated in gonadal cell lines by exogenous gonadotropins. However, the exact mechanism of GATA-4 mediated cell proliferation is not known (Heikinheimo et al., 1997; Vaskivuo et al., 2001). Targeting GATA-4 using RNA interference mechanisms in combination with standard chemotherapy may be useful in treating ovarian cancer.

**Prostaglandin F2-α receptor**

It shows pro-apoptotic effects in the ovary. It is expressed in the corpus luteum, thecal cells and interstitial cells. It is however, absent in granulosa cells at all stages of follicular development and also in oocytes. It is involved in apoptosis in the corpus luteum of during luteolysis. It upregulates endothelin-1 expression which plays a role in luteolysis (Moeljono et al., 1977; Orlicky et al., 1992; Girsh et al., 1996).

**Endothelins**

These are protein molecules that function in vasoconstriction, mitogenesis and steroid production (Luscher and Barton, 2000). FSH is known to exert its effects by stimulating cyclic-AMP action. FSH regulates Endothelin-1 levels in a dose-dependent manner (Otani et al., 1996). Endothelins abrogate the action of cyclic AMP mediated by FSH. ET-1 is over-expressed in the corpus luteum during luteolysis and is thought to have pro-apoptotic effects in the corpus luteum (Otani et al., 1996).

**Integrins**

that connect the cell to the cytoskeleton, and are able to influence cell survival and cell death (Aoudjit and Vuori, 2012). They function in cell proliferation via signal transduction pathways by activating protein kinases (Giancotti and Ruoslahti, 1999). Integrins are expressed in primordial follicular cell surfaces, aiding their adhesion to the extracellular matrix. Integrins are weakly expressed in atretic tertiary follicles and absent in atretic primary and secondary follicles. Granulosa cells that lack the expression of integrins were shown to be the only ones that undergo apoptosis (Giebel et al., 1996). Ovarian tumour growth and angiogenesis were shown to be inhibited when volociximab, a chimeric monoclonal antibody was directed against α5β1-integrin (Sawada et al., 2008). Intetumumab (CNT095) is a humanized anti-integrin monoclonal antibody that targets and binds with high affinity (Kd, approximately 1–24 nmol/L), to cells expressing αv integrin. Intetumumab has been shown to inhibit cell adhesion, invasion, migration, and proliferation of tumour and endothelial cells in vitro. Intetumumab also reduced tumour metastasis in vivo in nude mice with human breast cancer xenografts by inactivating the focal adhesion kinase (FAK) and the docking protein paxillin (Ning et al., 2010). Intra-peritoneal administration Etaracizumab, a humanized antibody, in SKOV3ip1 and HeyA8 mouse model ovarian cancer tumours targeting the αvβ3 integrin receptor decreased tumour burden by 36 and 48% respectively (Landen et al., 2008). However, its role in ovarian cancer patients is still to be exploited in clinical trials. Cilengitide, an αvβ3 and αvβ5 integrin inhibitor, is a cyclic RGD containing pentapeptide. Perry et al. (2013), using breast cancer cell lines (MCF-7 cells and then MDA-MB-231) treated with Cilengitide has shown that it was able to induce apoptosis and arrest cell proliferation. In another study, a combined approach with cilengitide and radiotherapy in breast cancer cells showed higher efficacy than either treatments alone (Lautenschlaeger et al., 2013). This study together with others have actually supported the idea that cilengitide in combination with radiation can be useful tool against ovarian cancer associated with integrins.

**Insulin-like growth factor (IGF)**

IGFs are proteins with high sequence similarity to insulin that stimulate mitosis; regulating cell proliferation, differentiation and apoptosis. IGF binding proteins regulate the activity of IGFs by modulating IGF binding to their cognate receptors (Werner et al., 2008). IGF-1 functions in the ovary by potentiating the action of gonadotrophic hormones. IGF-1 stimulates and sustains signals that lead to cell proliferation and inhibition of apoptosis by activation of the P13K/AKT pathway (Zheng et al., 2002). A high IGF-1 expression in mice follicles suggests that it plays an important role in follicle development. The roles of IGF binding proteins may differ; while IGFBP-4 is highly expressed in atretic follicles, IGFBP-5 is up-regulated in healthy primary and secondary follicles (Besnard et al., 1996). IGF over-
expression correlates with poor disease prognosis in some cases of ovarian carcinomas (Brokaw et al., 2007).

Small molecule inhibitors impede IGF-IR activation by binding to the ATP-binding pocket of the receptor. Insulin receptor signaling can be attenuated by most tyrosine kinase inhibitors. Despite this side effect due to lack of specificity, they showed activity in preclinical models and some are being evaluated in clinical trials. A potent insulin-like growth factor type 1 receptor (IGF-1R) inhibitor, NVP-AEW541, was shown to sensitize ovarian cancer cells to the effect of cisplatin (Beauchamp et al., 2010). In addition to apoptosis induction, it was found that AKT activation was also decreased by NVP-AEW541 (Beauchamp, et al., 2010) in two human epithelial ovarian cancer cell lines, namely, OVCAR-3 and OVCAR-4. Another molecule exploited is, BMS-536924 which is a potent small molecule inhibitor of IGF-IR, which shows antitumor activity in multiple tumour models. It has been shown to induce apoptosis in ovarian cancer cells by activating the cleavage of Poly ADP Ribose Polymerase (PARP) (Beauchamp et al., 2009). This shows that co-administration of BMS-536924 and a PARP inhibitor might be an effective strategy to curb resistance in ovarian cancer. In another study by Beltran et al. (2014) using AMG 479, a fully human monoclonal antibody against IGF-1R, they reported that AMG479 served as second line therapy in patients with recurrent platinum-sensitive ovarian cancer by blocking the binding of IGF1 and IGF2. There is a possibility that these agents might be more potent anticancer drugs since insulin receptor present on malignant cells may have an important role as well in carcinogenesis (Figure 1.)

Central to this mechanism, is the activity of Gonadotropins and other upregulated proteins such as Aurora A, PARP, mammalian target of rapamycin (mTOR) and growth factors such as EGFR, VEGFR, and Platelet-derived growth factor receptors (PDGFR) which have been exploited as molecular targets in various types of epithelial ovarian cancer and are at various phases in clinical trials. The numbered red stars represent various molecular targets that have been explored in ovarian cancer. Aurora A is a potent prosurvival molecule that exerts its effects by activating AKT, promoting DNA repair by activating BRCA1, blocking p53 activity, and activating the transcription factor NFkB. Growth factor receptors [EGFR (4), VEGFR (5), and PDGFR (6)] which exert...
Table 1. A table showing small molecule inhibitors/ monoclonal antibodies and their molecular targets in ovarian cancer.

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<tr>
<th>Small molecule drug (generic name)</th>
<th>Molecular target</th>
<th>Clinical trial phase</th>
<th>References</th>
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<td>Tozasertib (MK-0457)</td>
<td>Aurora kinase</td>
<td>Phase I</td>
<td>Sun et al. (2007), Lin et al. (2008) and Traynor et al. (2011)</td>
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<tr>
<td>Nultin-2</td>
<td>MDM2</td>
<td>Phase I</td>
<td>Wang et al. (2012)</td>
</tr>
<tr>
<td>Olaparib</td>
<td>PARP (Poly-ADP ribose)</td>
<td>Phase I</td>
<td>Fong et al. (2008)</td>
</tr>
<tr>
<td>Erlonitib and gefitinib</td>
<td>EGFR (epidermal growth factor receptor)</td>
<td>Phase II (gefitinib), Phase III (erlonitib)</td>
<td>Schilder et al. (2005) and Vergote et al. (2013)</td>
</tr>
<tr>
<td>BMS-690514</td>
<td>VEGFR (vasculo-endothelial growth factor receptor)</td>
<td>Not yet entered clinical trials for ovarian cancer</td>
<td>Becker et al. (2013)</td>
</tr>
<tr>
<td>Imatinib mesylate (STI57)</td>
<td>PDGFR (Platelet-derived growth factor receptor)</td>
<td>Phase II</td>
<td>Alberts et al. (2007)</td>
</tr>
<tr>
<td>Everolimus (RAD001)</td>
<td>mTOR</td>
<td>Phase I</td>
<td>Mabuchi et al. (2007) and Okamoto et al. (2010)</td>
</tr>
<tr>
<td>APR-246</td>
<td>P53</td>
<td>Phase I</td>
<td>Von Euler et al. (2014)</td>
</tr>
<tr>
<td>LBW242</td>
<td>XIAP</td>
<td>Phase I</td>
<td>Eschenburg et al. (2012) and Petrucci et al. (2012)</td>
</tr>
<tr>
<td>Nil</td>
<td>Integrin</td>
<td>Not entered clinical trials for ovarian cancer</td>
<td>Landen et al. (2008)</td>
</tr>
<tr>
<td>BMS-536924, NVP-AEW541, Gantutam (AMG-479)</td>
<td>IGFR1</td>
<td>Not entered clinical trials for ovarian cancer Phase I</td>
<td>Beuchamp et al. (2009, 2008) and Beuchamp et al. (2010)</td>
</tr>
<tr>
<td>ABT-737</td>
<td>Bcl-2/Bcl-Xl</td>
<td>Phase I</td>
<td>Witham et al. (2007) and Jain et al. (2014)</td>
</tr>
</tbody>
</table>

prosurvival effects via the P13K/AKT pathway have been targeted in ovarian cancer. XIAP, which can be upregulated by growth signals from NFkB is the most potent known inhibitor of caspases and has also been targeted using small molecules. Intergins such as α5β1-integrin exert prosurvival effects by activating protein kinases. IGF-1 functions in the ovary by potentiating the action of gonadotrophic hormones. IGF-1 stimulates and sustains signals that lead to cell proliferation and inhibition of apoptosis by activation of the P13K/AKT pathway. MDM2 which interacts with RBBP6 to promote p53 ubiquitination has been targeted as well with Nultin-2 (Table 1).

**Tyrosine kinases inhibitors**

Tyrosine kinases are molecules that play crucial roles in signal transduction, culminating their effects by regulating gene transcription within the nucleus. They function by transferring γ-phosphate groups from adenosine triphosphate to the hydroxyl group of protein molecules responsible for signal transduction (Schlessinger, 2000). A major event that activates tumour proliferation is the phosphorylation of signal transduction molecules. The most essential cellular processes such as the cell cycle, cell division, differentiation, motility and apoptosis or cell survival are under scrupulous regulation of tyrosine kinases (Prenzel et al., 2001; Slichenmyer and Fry, 2001). A dysfunction in tyrosine kinases pre-dispose signal transduction molecules to sustained phosphorylation; hence abnormal cell proliferation. Tyrosine kinases have been found over-expressed or mutated in several types of tumours in humans including ovarian tumours (Levitzki and Gazit, 1995; Blume-Jensen and Hunter, 2001; Wiener et al., 2003) making them good targets for cancer therapy. Small molecules have been developed to target tyrosine kinases in ovarian cancer.

**Angiogenesis inhibitors**

One of the key features leading to metastasis and invasion of normal tissues by cancerous cells is angiogenesis (the formation of new blood vessels). Poor prognosis in ovarian cancer has been confirmed to be associated with increased expression of the vascular endothelial growth factor (VEGF) which has functions such as angiogenesis, mitogenesis, improvement of vascular permeability, and endothelial cell survival (Folkman, 1997; Hartenbach et al., 1997). Small molecule tyrosine kinase inhibitors have been developed to target the VEGF ligand and the VEGF receptor in ovarian cancer thereby slowing angiogenesis and improving upon prognosis of disease (Friedlander et al., 2007; Campos et al.,
Inhibitors of the epidermal growth factor receptor (EGFR)

EGFR is a transmembrane tyrosine kinase protein receptor that is involved in cell proliferation, survival and differentiation (Herbst, 2004). About 70% of ovarian cancers show upregulated expression in EGFR, making it an attractive target for the treatment of ovarian carcinomas. Over-expression has been shown to correlate with chemoresistance and poor prognosis. Increased cell proliferation, angiogenesis and reduced apoptosis are attributed to over-expression of EGFR (Bartlett et al., 1996; Fischer-Colbrie et al., 1997). Tyrosine kinase inhibitors such as erlotinib and gefitinib have been successfully directed against the EGFR (Sirotnak et al., 2000; Sirotnak, 2003). Phase 2 clinical trials with gefinitib in patients with advanced recurrent ovarian carcinomas showed very little activity, although the drug was well tolerated (Schilder et al., 2005). Phase 3 trials with erlotinib, however did not show any significant improvement in activity (Vergote et al., 2013).

Aurora kinase inhibitors

Aurora A is a serine-threonine kinase that is required for many essential cellular functions such as mitosis, spindle formation and centromere separation (Bischoff et al., 1998; Campos and Gosh, 2010). An over-expression of Aurora A as well as amplification of its gene location have been frequently noted in human tumours, including in ovarian carcinomas (Bischoff et al., 1998; Zhou et al., 1998; Lingle et al., 1998; Landen et al., 2007). Aurora A has been shown to inhibit paclitaxel and cisplatin – mediated apoptosis in ovarian cancer cells (Yang et al., 2006; Anand et al., 2003). Aurora kinase inhibition with a small molecule MK-0457 in combination with chemotherapy (docetaxel) has shown significant reduction in tumour growth and cell proliferation in HeyA8 and SKOV3ip1 ovarian cancer cell lines (Sun et al., 2007; Lin et al., 2008; Traynor et al., 2011).

Poly ADP Ribose Polymerase (PARP) inhibitors

BRCA1 and BRCA2 play an important role in DNA double strand break repairs and maintaining genomic stability (Lord and Ashworth, 2008). BRCA ovarian cancer patients show an impaired ability to repair damaged DNA. Mutations in these genes account for 5 to 10% of all ovarian cases. BRCA genes represent the most important risk factor in ovarian cancer with lifetime risks of between 40 to 50% and 10 to 20% for BRCA1 and BRCA2 respectively (Cannistra, 2004; Lord and Ashworth 2008). More than 50% of high grade serous ovarian carcinomas show loss of function of BRCA genes, either by genetic or epigenetic causes (Press et al., 2008). Poly ADP Ribose Polymerase (PARP) is a nuclear enzyme involved in the DNA single strand break repair (Tutt et al., 2005). It is activated in DNA damage and its inhibition results in DNA single strand breaks which may result in double strand breaks. BRCA1 and BRCA 2 patients show high sensitivity to DNA-damaging chemotherapeutics and have also shown immense responsiveness to PARP inhibitors (Bryant et al., 2005; Tutt et al., 2005). Although the mechanism is not fully understood, Lord and Ashworth (2008) suggest that an excessive amount of DNA single strand breaks with subsequent double strand breaks leads to high irreparable genomic instability and hence, cell death. Olaparib is a small molecule PARP inhibitor that has been used and has shown efficacy in BRCA1 ovarian cancer patients and has passed the first phase of clinical trials (Fong et al., 2008).

Platelet-derived growth factor (PDGF) receptor inhibitors

PDGF is involved in cellular growth, survival, differentiation, vascular permeability, cellular migration and healing of wounds (Schmitt and Matei, 2008). Between 50 and 80% of ovarian cancers show activation of the PDGF receptor which is involved in neoplastic transformation (Heinrich et al., 2003; Apte et al., 2004). PDGF receptor activation is as a result of mutations, genetic amplification or chromosomal rearrangements (Carroll et al., 1996; Heinrich et al., 2003). Imatinib mesylate (STI57) is a small molecule that has been used to target PDGF receptor. A significant induction of apoptosis and reduction in tumour weight was observed in this study when STI571 was used in combination with taxol. However, STI571 alone did not cause any significant effects (Apte et al., 2004). Imatinib is a small molecule PDGF receptor inhibitor that has passed phase II clinical trials in ovarian cancer patients whose tumours express the PDGF receptor (Alberts et al., 2007).

MTOR inhibitors

PTEN is a lipid phosphatase that is involved in G1 cell cycle arrest and apoptosis through the AKT /PI3K /mTOR pathway, and has been shown to be mutated, deleted or inactivated in gynaecologic tumours (Sansal and Sellers, 2004; Jiang and Liu, 2008; Campos and Gosh, 2009). The mTOR pathway is a key regulator of cell growth, proliferation and programmed cell death (Campos and Gosh, 2009). Inhibitors of mTOR such as everolimus (RAD001) have been shown to inhibit angiogenesis, tumour proliferation and ascites formation in vivo and in vitro using OVCA10 and SKOV-3 ovarian cancer cells. It is also improved upon cisplatin-mediated apoptosis. These
suggest a promising role of mTOR inhibitors to treat ovarian tumours (Gera et al., 2004; Mabuchi et al., 2007; Okamoto et al., 2010).

CONCLUSION

Just as in other tumours in humans, a detailed understanding of the molecular abnormalities in the various types of ovarian tumours seems to be the only means to maximize the positive therapeutic effects and better prognosis derived from targeted therapy. Similar genetic and histopathological characteristics exist in tumours of the same sub-type (Gilks, 2009).

However, our comprehension of genetic and molecular aberrations including the mechanisms of oncogenic transformation seems to be at its infancy; given that even with targeted therapeutic treatments in combination with chemotherapy, a high proportion of patients still develop recurrent disease and subsequently, death. Apoptosis remains the most important mechanism of cell death in cancer therapy. However, for apoptosis to be selectively induced as a therapeutic measure, the ideal targets need to be found. A diligent search of other molecular targets and an improved understanding of the pathways involved are essential for us to maximize the benefits of targeted therapy.

The genetic and molecular characteristics of ovarian tumours are not the same, even in the same ovarian tumour subtype. The more understanding we achieve, the better the prognosis of ovarian cancer, and the more individualized targeted therapy would become.

Conflict of interest

The authors declare that they have no interest.

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