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Dysregulated TGFβ Signaling in Ovarian Cancer

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1. Introduction

Ovarian cancer is one of the most lethal gynecological cancers in the United States. NCI estimates ~21,880 new cases with ~13,850 deaths in 2011 (http://www.cancer.gov/ cancertopics/ types/ovariancancer). Unfortunately, the majority of these cases are only discovered at advanced stages (stage III or IV) due to the cancer's asymptomatic nature which has an overall survival rate between 5-25% (Bast et al., 2009; Hennessy et al., 2008). Hence, the inability to detect this disease during early stages has led to poor prognosis. Despite improvements in medicine and patient care, reasonable screening measures for detecting early stage ovarian cancers are presently lacking. Thus, a better understanding of the molecular events that underlie ovarian cancer development are needed.

The current strategy for treatment of ovarian cancer is surgical debulking followed by chemotherapy (Bast et al., 2009; Hennessy et al., 2008). Although ~70% of ovarian cancers respond to a combination of platinum and taxane-based chemotherapy administered after surgery, current treatments are of limited efficacy in preventing tumor recurrence and progression (Bast et al., 2009; Hennessy et al., 2008). Thus, new anti-neoplastic agents are urgently needed to increase the chemotherapeutic sensitivity of ovarian cancer cells.

Recently, evidence has emerged revealing the importance of genomic aberrations in the progression of ovarian cancer (Gorringe & Campbell, 2009; Gray et al., 2003). Through the use of high throughput technologies (i.e. array comparative genomic hybridization (aCGH), microarray, and SNP arrays), specific genomic regions have been identified to be either amplified or silenced in tumor progression (Gorringe & Campbell, 2009; Gray et al., 2003). One such region which we and others (Nanjundan et al., 2007; Osterberg et al., 2009) have previously identified to be frequently amplified early in serous epithelial ovarian cancer development is the 3q26.2 region which harbors Transforming Growth Factor β pathway (TGFβ) co-repressors, ecotropic viral integration site-1 (EVI1) (Nanjundan et al., 2007) and SnoN/SkiL (Nanjundan et al., 2008). A large amount of work has recently emerged involving the intricacies of TGFβ signaling and its role in cancer progression. Importantly, this signaling pathway is dysregulated in ovarian carcinomas.

2. Dual functionality of TGFβ signaling in cancer

There exist three isoforms of TGFβ, namely TGFβ1, TGFβ2, and TGFβ3, which are initially present in the inactive latent form (L-TGFβ) (Elliott & Blobe, 2005; Meulmeester & Ten Dijke,

2011). In its active dimeric form, the TGFβ ligand binds to the TGFβ receptor type II (TGFβRII) leading to heterotetrameric receptor complex formation with TGFβRI. In addition, the co-receptor, TGFβRIII or proteoglycan (a.k.a. endoglin), aids binding of the ligand to the TGFβRII (Elliott & Blobe, 2005; Meulmeester & Ten Dijke, 2011). The activated receptors then recruit receptor regulated SMADs (R-SMADs) such as SMAD2/3 which form a complex with a Co-SMAD, SMAD4, and then shuttles into the nucleus. These activated SMADs associate with DNA binding transcription factors to enhance DNA binding to regulate transcription of TGFβ target genes such as cyclin-dependent kinase inhibitors (i.e. p21, involved in regulating cell survival) (Elliott & Blobe, 2005) (Figure 1). The TGFβ pathway is regulated via several mechanisms including (1) phosphorylation, (2) ubiquitination, (3) inhibitory SMADs (i.e. SMAD6 and SMAD7), and (4) transcriptional co-repressors (i.e. SnoN/SkiL and EVI1) (Elliott & Blobe, 2005; Meulmeester & Ten Dijke, 2011). In addition to the canonical SMAD dependent pathway, there exists the non-canonical pathway involving (1) TRAF5/TAK1/p38-JNK, (2) RhoA/ROCK, and (3) ERK/MAPK (Elliott & Blobe, 2005; Meulmeester & Ten Dijke, 2011).
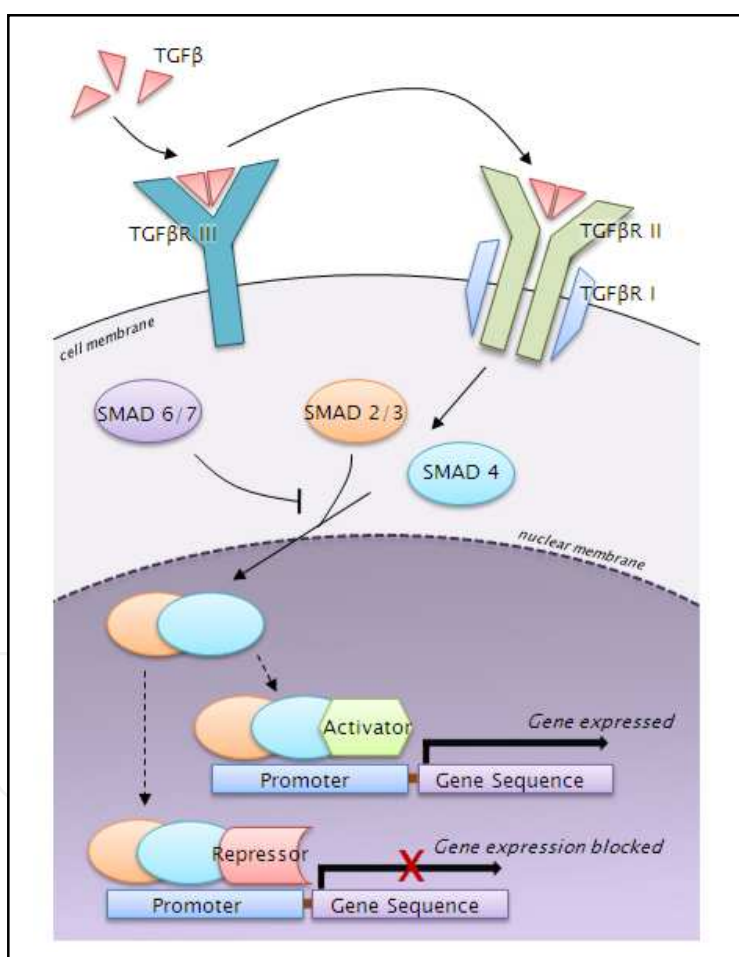

Fig. 1. The TGFβ signaling pathway. Active dimeric TGFβ ligand binds to TGFβRII on the cell surface leading to complex formation with TGFβRI. Endoglin (TGFβRIII) assists in recruitment of the active TGFβ ligand to bind to the cell surface receptors. Following receptor activation, receptor SMADs (SMAD2/3) become phosphorylated and form a complex with the Co-SMAD (SMAD4) which then translocate into the nuclear compartment to regulate transcription of various TGFβ target genes.

The TGFβ signaling pathway has the ability to transition from a tumor suppressor (in normal or early stage cancers) to a tumor promoter (late stages of cancer) (Elliott & Blobe, 2005; Meulmeester & Ten Dijke, 2011) (Figure 2). During the early stages of epithelial tumorigenesis, TGFβ inhibits tumor development and growth by inducing cell cycle arrest, senescence, and apoptosis; this aids in maintaining cellular homeostasis critical for prevention of continuous cell proliferation and thus tumor formation (Elliott & Blobe, 2005). This functionality is elicited via induction of cyclin-dependent kinase inhibitors (CDK), namely p15, p21, and p27. TGFβ also represses expression of the c-myc oncogene which leads to activation of these CDK inhibitors (Elliott & Blobe, 2005; Meulmeester & Ten Dijke, 2011). Additional molecules that are involved in the TGFβ apoptotic functional response include the death receptor FAS, GADD45b, BIM, and DAPK (Elliott & Blobe, 2005; Meulmeester & Ten Dijke, 2011).
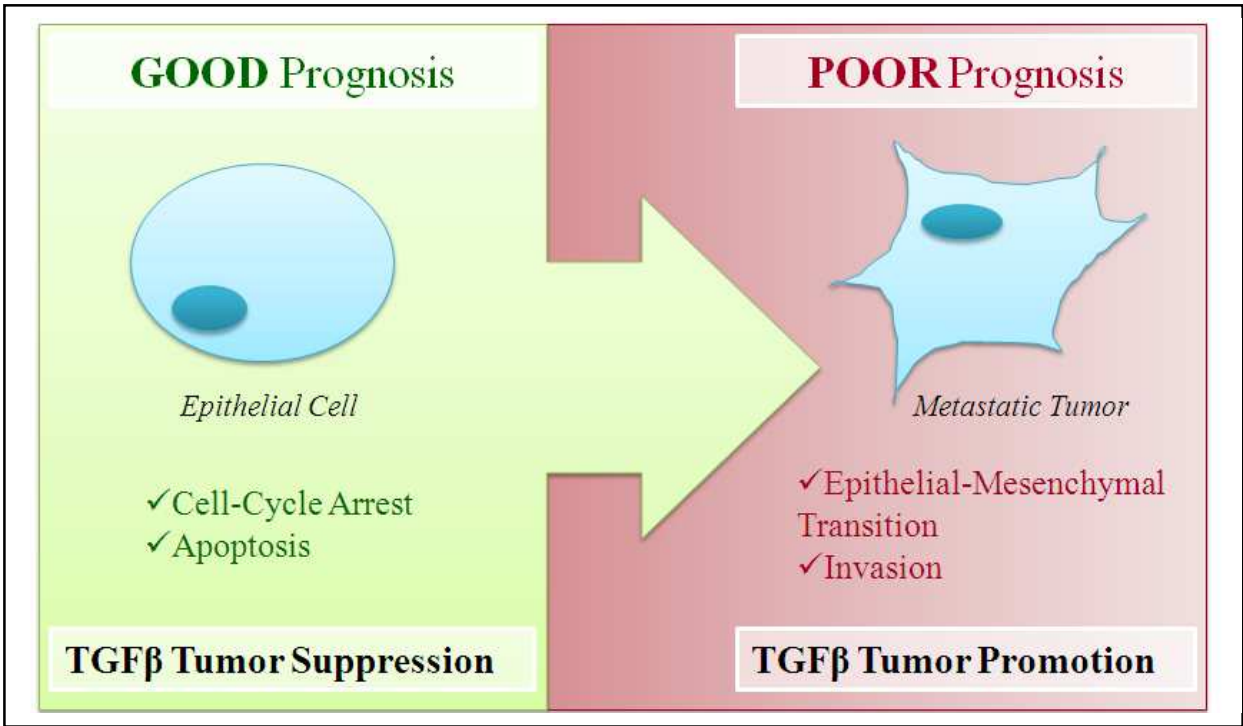

Fig. 2. The TGFβ signaling pathway elicits dual functionality. The pathway can transition from a tumor suppressive (normal or early stages of cancer) role to a tumor promoting role (late stages of cancer).

During the progression of cancer, mutations in these components may lead to disruption of TGFβ mediated control of cell proliferation. In late stages of tumor progression, tumor cells become resistant to growth inhibition due to inactivation of the TGFβ signaling pathway thus leading to altered cell cycle control (Elliott & Blobe, 2005). TGFβ becomes capable of inducing metastatic functions via increased cellular migration, invasiveness, loss of epithelial markers, and a corresponding acquisition of mesenchymal characteristics.

3. Dysregulated TGFβ signaling in ovarian cancer

Several components of the TGFβ signaling pathway have been reported to be dysregulated in ovarian cancers and are summarized in the subsections below.

3.1 TGFβ ligand expression in ovarian cancer

By immunohistochemical and in situ hybridization approaches, all of the three TGFβ ligands (TGFβ1, TGFβ2, and TGFβ3) are markedly elevated in ovarian cancer cells (Henriksen et al., 1995). Similar results were obtained via RNAse protection assay in primary ovarian cancer specimens (Bartlett et al., 1997). Northern blot analysis indicated that mRNA levels of both TGFβ1 and TGFβ3 are increased in recurrent ovarian cancers (Bristow et al., 1999). Using enzyme-linked immunosorbant assay (ELISA), TGFβ1 levels are increased in plasma and peritoneal fluid of advanced stage ovarian cancer patients (Santin et al., 2001). Increased expression of TGFβ appears to be correlated with a poor patient survival outcome which is associated with peritoneal metastasis, expression of vascular endothelial growth factor (VEGF), and microvessel density (markers of angiogenesis) (Nakanishi et al., 1997).

Mutational analysis of TGFβ1 assessed by PCR-SSCP (polymerase chain reaction single-strand conformational polymorphism) uncovered defects in the coding region of exons 5, 6, and 7 (Cardillo et al., 1997). However, these alterations were not associated with histological type of the tumor or its transcript/protein expression levels (Cardillo et al., 1997).

3.2 TGFβ receptor expression in ovarian cancer

There appears to be some discrepancy in the reported levels of TGFβ receptors in ovarian cancer which may be due to the nature of the cell lines and tumor specimens assessed. In one study, the proximal components of the TGFβ signaling pathway (receptor expression and its phosphorylation status) appeared to be intact in primary ovarian cancer cell cultures; this indicated that downstream mechanisms could be responsible for growth resistance to TGFβ such as increased matrix metalloproteinase-2 (MMP2) expression (Yamada et al., 1999). Yet in another report, TGFβRII transcripts were undetectable in TGFβ resistant ovarian cancer cell lines (AZ224 and AZ547) whereas SKOV3 cells were positive for TGFβRII expression (Zeinoun et al., 1999). TGFβRII was also detectable in an additional 14 ovarian cancer cell lines (Hu et al., 2000) (Xi et al., 2004). A more recent study reported reduced TGFβRII levels which was determined via microarray analysis and validated via real-time PCR (qPCR) (Sunde et al., 2006).

Via northern blot analysis, expression of TGFβRI and TGFβRIII was markedly reduced in recurrent ovarian tumors (Bristow et al., 1999). In an independent study, TGFβRIII was notably decreased or absent in ovarian cancers at the RNA and protein levels (Hempel et al., 2007).

Mutational analysis of TGFβRI and TGFβRII uncovered mutations in a minority of ovarian cancers (Ding et al., 2005). Specifically, a frameshift mutation has been identified in Exon 5 of TGFβRI in 31% of ovarian tumors (Wang et al., 2000b), in exons 2, 3, 4, and 6 of TGFβRI (catalytic domain of the kinase) in 33% primary ovarian cancers (Chen et al., 2001), and deletions in exon 1 of TGFβRI in <30% of ovarian tumors (Antony et al., 2010). Likewise, missense mutations have been identified in TGFβRII (Francis-Thickpenny et al., 2001) and deletions in exon 3 of TGFβRII in ovarian tumors (Antony et al., 2010).

3.3 SMAD expression in ovarian cancer

Decreased expression of SMAD4 has been described in several ovarian cancer cell lines (Hu et al., 2000) which appears to correlate with dysregulated expression of p21 and c-myc (Antony et al., 2010).

Unlike pancreatic cancer in which ~50% of SMAD4 is mutated (Elliott & Blobe, 2005), reports of the presence of SMAD4 variants in ovarian cancers are lacking (Wang et al., 2000b). However, mutational analysis of additional SMAD family members showed that 35% and 23%, respectively, of ovarian tumor specimens contained a polymorphism in intron 2 of SMAD6 and a polymorphism at codon 208 in SMAD7. Neither of these mutations were associated with amino acid changes and thus, are unlikely to be important in ovarian cancer development (Wang et al., 2000a). Similarly, 42% of ovarian tumor specimens had a polymorphism for SMAD2 which was not associated with an amino acid change; thus it is also unlikely that this mutation is significant in the development of ovarian cancer (Wang et al., 1999).

3.4 TGFβ transcriptional co-regulator/co-factor expression in ovarian cancer

Ecotropic viral integration site-1 (EVI1), a TGFβ corepressor, was elevated up to 40-fold in ovarian carcinoma cells via RNAse protection assay (Brooks et al., 1996). Similarly, via microarray analysis and qPCR validation, EVI1 was found to be upregulated in advanced stage ovarian cancers (Sunde et al., 2006). In our analysis, we identified that EVI1 and MDS1/EVI1 are amplified in advanced stage serous epithelial ovarian cancers at the DNA, RNA, and protein levels via aCGH, transcriptional profiling/qPCR analysis, and western blot analysis (Nanjundan et al., 2007). Further, SnoN/SkiL, another TGFβ corepressor, is likewise increased at the DNA and RNA levels (cCGH and qPCR) in advanced stage serous epithelial ovarian cancers (Nanjundan et al., 2008). In addition, c-myc, an oncogenic transcriptional regulator, is upregulated in ovarian cancers (Garte, 1993).

3.5 Other TGFβ mediator expression in ovarian cancer

Other TGFβ mediators whose expression is altered in ovarian cancers include DACH1 and BMP7 which are both upregulated and inhibit TGFβ signaling (Sunde et al., 2006). Mediators in ovarian cancers that are downregulated include PCAF and TFE3 (which enhance TGFβ signaling) (Sunde et al., 2006). Other molecules that could attenuate TGFβ proliferative control include FOXG1 which is overexpressed in high-grade ovarian cancers and suppresses p21 WAF1/CIP1 transcription (Chan et al., 2009). BAMBI (BMP and activin membrane-bound inhibitor) is overexpressed in ovarian cancers promoting resistance to TGFβ mediated apoptosis by shuttling into the nuclear compartment with SMAD2/3 in a TGFβ dependent manner (Pils et al., 2011). EZH2 is increased in ovarian cancers and appears to be involved in altering metastatic potential by upregulating TGFβ1 (Rao et al., 2010). A SMAD4 target gene, RunX1T1, is a tumor suppressor in ovarian cancers and is repressed by histone modifications (Yeh et al., 2011).

4. SnoN/SkiL, a TGFβ transcriptional modulator, in ovarian cancer

SnoN/SkiL belongs to the Ski family (i.e. Ski, SnoN, Fussel-15, and Fussel-18), a group of proto-oncogenes involved in early developmental processes sharing structural and

functional features characteristic of winged-helix/forkhead class of DNA binding proteins (Deheuninck & Luo, 2009). However, these proteins do not directly bind to DNA but associate to DNA via interaction via nuclear proteins (i.e. SMADs) (Deheuninck & Luo, 2009). Thus, the mechanism of repression of TGFβ signaling occurs by transcriptional modulation by recruitment of nuclear corepressors (i.e. N-CoR), histone deacetylase complex (HDAC), and interference of SMAD-mediated binding to the transcriptional coactivator, p300/CBP (Deheuninck & Luo, 2009). Ski and SnoN genes have an overall homology of 50% and both are tightly regulated at multiple levels via: (1) transcriptional regulation, (2) protein degradation, (3) post-translational modifications, and (4) subcellular localization (Deheuninck & Luo, 2009; Luo, 2004; Pan et al., 2009). SnoN is not only phosphorylated by TGFβ activating kinase (TAK1) but can also physically associate with TAK1 leading to SnoN degradation (Kajino et al., 2007). SnoN is degraded rapidly via proteasome-mediated degradation upon TGFβ stimulation via SMURF2, APC, and Arkadia (RNF111) E3 ubiquitin ligases (Inoue & Imamura, 2008; Izzi & Attisano, 2004; Levy et al., 2007). SnoN can also interact with promyelocytic leukemia protein (PML) which promotes its association with PML nuclear bodies to stabilize p53 leading to induction of premature senescence (Lamouille & Derynck, 2009; Pan et al., 2009). SnoN can be sumoylated via SUMO E3 ligase PIAS independently of TGFβ signaling and its ubiquitination status (Hsu et al., 2006; Wrighton et al., 2007). Although sumoylation does not alter its stability or subcellular localization, it may augment SnoN-mediated repression of TGFβ signaling on specific promoters such as the myogenin promoter (Hsu et al., 2006). Although SnoN is predominantly nuclear localized, it can be cytoplasmically localized in normal cells under non-pathological conditions (Krakowski et al., 2005).

Both Ski and SnoN are expressed in all adult tissues at low levels and are involved in differentiation of neural and muscle cells (Deheuninck & Luo, 2009; Luo, 2004; Pan et al., 2009). Expression of Ski and SnoN are altered in numerous disease states including cancer (Deheuninck & Luo, 2009; Luo, 2004; Pan et al., 2009). Our research indicates that SnoN levels are upregulated in serous epithelial ovarian cancers via different mechanisms including gene amplification, altered protein stability, and transcriptional activation (Nanjundan et al., 2008). Further, siRNA targeting SnoN leads to reduction in ovarian cancer cell proliferation implicating a pro-oncogenic function (Nanjundan et al., 2008; Smith et al., 2010). In addition, attenuated SnoN protein via siRNA is detrimental to breast and lung cancer cellular transformation in both *in vitro* and *in vivo* mouse xenograft models (Zhu et al., 2007). Strikingly, SnoN has also been implicated in a tumor suppressive function. Deletion of one copy of SnoN leads to increased susceptibility to carcinogen-induced tumor development (Deheuninck & Luo, 2009; Luo, 2004; Pan et al., 2009). Furthermore, long-term stable expression of SnoN in an ovarian cell line leads to induction of senescence (i.e. oncogene-induced senescence similar to that described for Ras) (Nanjundan et al., 2008). In another study, SnoN induces premature senescence in a PML and p53–dependent fashion; it also inhibits epithelial-mesenchymal transition (EMT) and tumor metastasis in breast and lung cancer cells (Pan et al., 2009; Zhu et al., 2007). Collectively, these findings suggest that SnoN elicits multiple roles in cancer development.

5. EVI1, a TGFβ transcriptional modulator, in ovarian cancer

EVI1, ectopic viral integration site-1 protein, now called MECOM (MDS1 and EVI1 complex) is located at the 3q26.2 locus. It was initially identified as a site for viral integration

in mouse cancer models; it has been well studied as an oncogene in acute myeloid leukemia (AML) and in myelodysplastic syndrome (MDS) (Levy et al., 1994; Morishita et al., 1992b). Functions of EVI1 include (1) proliferation of leukemic cells (Tanaka et al., 1995), (2) cellular transformation (Kilbey & Bartholomew, 1998), (3) inhibition of growth factor mediated differentiation and survival (Morishita et al., 1992a), (4) induction of neural and megakaryocyte differentiation, and (5) inhibition of interferon (Buonamici et al., 2005) and TGFβ signaling (Izutsu et al., 2001; Soderholm et al., 1997; Sood et al., 1999; Vinatzer et al., 2003; Vinatzer et al., 2001). Notably, EVI1 represses transcription via binding to SMADs and recruiting CtBP1/HDAC (Izutsu et al., 2001; Palmer et al., 2001; Senyuk et al., 2002) to target promoter elements, increasing AP-1 activity (Tanaka et al., 1994), disrupting JNK induced apoptosis (Maki et al., 2008), inhibiting PML function (Buonamici et al., 2005), binding to BRG1 (Chi et al., 2003), and activating PI3K by reducing TGFβ and drug induced apoptosis (Liu et al., 2006; Yoshimi et al., 2011). Supporting its role as an inducer of cellular proliferation, EVI1 knockout mice are embryonically lethal due to hypocellularity across multiple organ sites (Hoyt et al., 1997). There exist multiple splice variants of EVI1 whose functions are presently unclear (Alzuherri et al., 2006; Jazaeri et al., 2010; Vinatzer et al., 2003). In particular, the MDS1/EVI1 is a read-through splice form which contains a novel PR (PRD1-BF1-RIZ homology) domain; its functionality is unclear and is suggested to be context or cell type dependent (either eliciting functionality similar or antagonistic to EVI1 (Vinatzer et al., 2003). Structurally, EVI1 contains 2 zinc finger domains, an intervening region required for transformation, and a repressor domain necessary for binding to CtBP1/HDAC (Nanjundan et al., 2007).

In ovarian cancer, the first report of altered EVI1 expression in ovarian carcinoma cells demonstrated up to a 40-fold increase in its mRNA levels via RNAse protection assay compared to the normal ovary; these initial findings implicate a novel role for EVI1 in solid tumor carcinogenesis (Brooks et al., 1996). A decade later, increased EVI1 levels in advanced stage ovarian cancers supported these initial findings via oligonucleotide arrays profiling and validation via qPCR analysis (Sunde et al., 2006). The same researchers also found that the EVI1 gene locus was amplified in 43% of the tumors with a significant correlation between gene copy and EVI1 gene expression levels (Sunde et al., 2006). They also reported that EVI1 inhibited TGFβ signaling in normal immortalized ovarian epithelial cells (Sunde et al., 2006). Our research has also uncovered increased copy number at the EVI1 locus in advanced stage serous epithelial ovarian carcinomas via aCGH analysis (Nanjundan et al., 2007). We found that EVI1 DNA copy number increases were associated with at least a 5-fold increase in RNA transcript levels in the majority of advanced ovarian cancers (Nanjundan et al., 2007). More recent whole genome aCGH analysis of stage III ovarian serous carcinomas also identified a gain at 3q26.2 with their gene expression analysis demonstrating elevated EVI1 expression (Osterberg et al., 2009). Protein level determination via western blotting analysis showed a corresponding increase in MDS1/EVI1 and EVI1 expression in ovarian cancers and multiple ovarian cancer cell lines (Nanjundan et al., 2007). Interestingly, functional studies by transient transfection into normal immortalized epithelial cells demonstrated that EVI1 and MDS1/EVI1 increased cell proliferation, migration, and decreased TGFβ-mediated plasminogen activator inhibitor-1 (PAI-1) promoter activity (Nanjundan et al., 2007). In yet another recent study, highest expression of EVI1 and a splice variant, Del324 (EVI1s), was observed in ovarian cancer specimens with a constant ratio between the two splice

variants across all specimens assessed (Jazaeri et al., 2010). However, their analysis did not identify an altered expression protein pattern between serous ovarian cancers and fallopian tube fimbria or benign neoplasms (Jazaeri et al., 2010). In support of our functional studies in OVCAR8 cells (Nanjundan et al., 2007), when EVI1 was expressed exogenously in this ovarian carcinoma cell line (which harbors a deletion at the EVI1 locus), there was no altered proliferation (Jazaeri et al., 2010). Furthermore, with knockdown of specific EVI1 forms (via siRNA and shRNA) in ovarian cancer cells, there was no alteration in functionality (Jazaeri et al., 2010). Although their data do not support a role for EVI1 in ovarian cancer cell proliferation (Jazaeri et al., 2010), further investigations are warranted to determine the functional relevance of disrupted TGFβ signaling via EVI1 in ovarian cancer.

5.1 Epigenetic aberrations, EVI1, and ovarian cancer

Epigenetic modifications refers to changes in gene expression as a result of DNA methylation, histone modification, nucleosome repositioning, and post-transcriptional gene regulation by micro-RNAs (Balch et al., 2009). DNA methyltransferases are involved in adding methyl groups to the cytosine-5 position within CpG dinucleotides (Balch et al., 2009). CpG dense regions, however, are normally unmethylated in normal specimens (Balch et al., 2009). Histone modifications are extensive and can regulate transcription in an open or closed conformation on the chromatin structure (Balch et al., 2009). These regions can be extensively altered in disease states such as cancer with a general DNA hypomethylation status and localized hypermethylation of promoter associated CpG islands in cases of tumor suppressor genes (Balch et al., 2009). Further, dysregulation of miRNA expression has been also linked to cancer development (Balch et al., 2009). A number of epigenetic aberrations are well noted in ovarian cancer (Balch et al., 2009).

Based on homology to proteins with PR domains, MDS1/EVI1 (which contains such a domain) has the potential to elicit protein methyltransferase activity (Vinatzer et al., 2003; Vinatzer et al., 2001). However, we did not detect any such activity associated with MDS1/EVI1 via *in vitro* methyltransferase activity assays using free histones as substrate (Nanjundan et al., 2007). There was some weak associated activity which we suggested to be due to co-immunoprecipitating molecules, possibly SWI/SNF components or proteins associated with methyltransferase activity (Nanjundan et al., 2007). Indeed, EVI1 has recently been shown to physically interact with molecules which have such activities (Cattaneo & Nucifora, 2008; Lugthart et al., 2011; Pradhan et al., 2011; Senyuk et al., 2011; Spensberger & Delwel, 2008).

Indeed, links between DNA hypermethylation and EVI1 are observed in AML (Lugthart et al., 2011); further, EVI1 physically interacts with DNA methyltransferase 3A/3B (DNMT3A/3B) (Senyuk et al., 2011). Thus, EVI1 is likely involved in promoter DNA methylation in leukemia and possibly in other solid tumors such as ovarian cancers. EVI1 regulates the expression of microRNA-124 which is involved in regulation of differentiation and cycling of hematopoietic cells (De Weer et al., 2011; Dickstein et al., 2010). This was demonstrated to occur via methylation of CpG dinucleotides upstream of the miRNA leading to its repression and hence, increased expression of genes involved in cell division such as Bmi1 and cyclin D3 (De Weer et al., 2011; Dickstein et al., 2010). Through its interaction with DNMT3, the EVI1 complex binds to regulatory regions of the miRNA to

regulate its expression (De Weer et al., 2011; Dickstein et al., 2010). Of further interest is the recent identification of the physical interaction between EVI1 and SIRT1, a histone deacetylase which is itself a direct target of EVI1. Interaction between SIRT1 and EVI1 leads to EVI1 degradation (Pradhan et al., 2011). SIRT1 is increased in AML patient samples where EVI1 is elevated (Pradhan et al., 2011). In addition, EVI1 interacts directly with SUV39H1 and G9a, both histone methyltransferases, which elicit methyltransferase activities and enhance the repressive activity of EVI1 (Cattaneo & Nucifora, 2008; Spensberger & Delwel, 2008). Thus, the oncogenic activity of EVI1 may be involved in deacetylation and methylation events which would lead to altered chromatin structure and, thus, transcriptional events.

6. Novel perspective into the functionality of TGFβ: Autophagy

More recently, TGFβ has been implicated in regulating autophagy (Gajewska et al., 2005; Kiyono et al., 2009), a self eating process whereby damaged cellular organelles and other cellular material are sequestered within autophagosomes. These double-membrane structures eventually fuse with single-membrane lysosomes leading to degradation of the inner contents (Huang & Klionsky, 2007; Yang & Klionsky, 2009) (Figure 3). Autophagy is activated in response to multiple stresses during cancer progression including nutrient starvation, the unfolded protein response (UPR), hypoxia, and cellular treatment with cytotoxic chemotherapeutic agents (Huang & Klionsky, 2007; Yang & Klionsky, 2009). It has been suggested that autophagy promotes tumorigenic development; thus, it would be an ideal target for tumor ablation. Indeed, increased levels of autophagy are observed in tumor cells following treatment of cells with chemotherapeutic agents (Kondo et al., 2005; Kondo & Kondo, 2006).

The isolation membrane of the autophagosome arises due to complex formation between beclin-1 and hVps34 (Geng & Klionsky, 2008; Klionsky, 2005; Wang & Klionsky, 2003; Yorimitsu & Klionsky, 2005). The membrane elongates via activation of ubiquitin-like conjugation system. ATG12 is activated by ATG7 which is then transferred to ATG10 and finally covalently attached to ATG5 (Geng & Klionsky, 2008). The ATG12-ATG5 conjugate localizes to autophagosome precursors and dissociates prior to or following completion of formation of the autophagic vacuole. Another ubiquitin-like modification system involving LC3 (microtubule associated protein 1 light chain 3) completes autophagosome formation (Geng & Klionsky, 2008). The cytosolic precursor of LC3 (LC3-I) becomes cleaved at its C-terminus by ATG4 and is conjugated to phosphatidylethanolamine (PE) to generate the membrane bound LC3-II form; this process requires ATG7 and ATG3 activities (Geng & Klionsky, 2008; Klionsky, 2005; Wang & Klionsky, 2003; Yorimitsu & Klionsky, 2005). LC3-II is specifically targeted to ATG12-ATG5 associated autophagosomal precursor membranes. Following fusion of autophagosomes with lysosomes, LC3-II becomes delipidated and returns to the cytosolic pool to be recycled (Geng & Klionsky, 2008; Klionsky, 2005; Wang & Klionsky, 2003; Yorimitsu & Klionsky, 2005).

The initial finding that TGFβ induces autophagy was observed in bovine mammary epithelial BME-UV1 cells; both LC3 and beclin-1 expression were induced following TGFβ1 treatment leading to cell death (Gajewska et al., 2005). Following reports support this finding in a number of cell lines including hepatocellular and breast carcinoma cell lines

(Kiyono et al., 2009). TGFβ was noted to induce autophagosome formation with a corresponding conversion of LC3-I to LC3-II and increased expression of autophagic markers including beclin-1, ATG5, ATG7, and DAPK (Kiyono et al., 2009). In addition, knockdown of SMADs and other targets in the non-canonical SMAD pathways decreased TGFβ mediated autophagy (Kiyono et al., 2009). Autophagy induction led to induction of BIM and BMF (proapoptotic markers) which occurred prior to initiation of apoptosis (Kiyono et al., 2009).
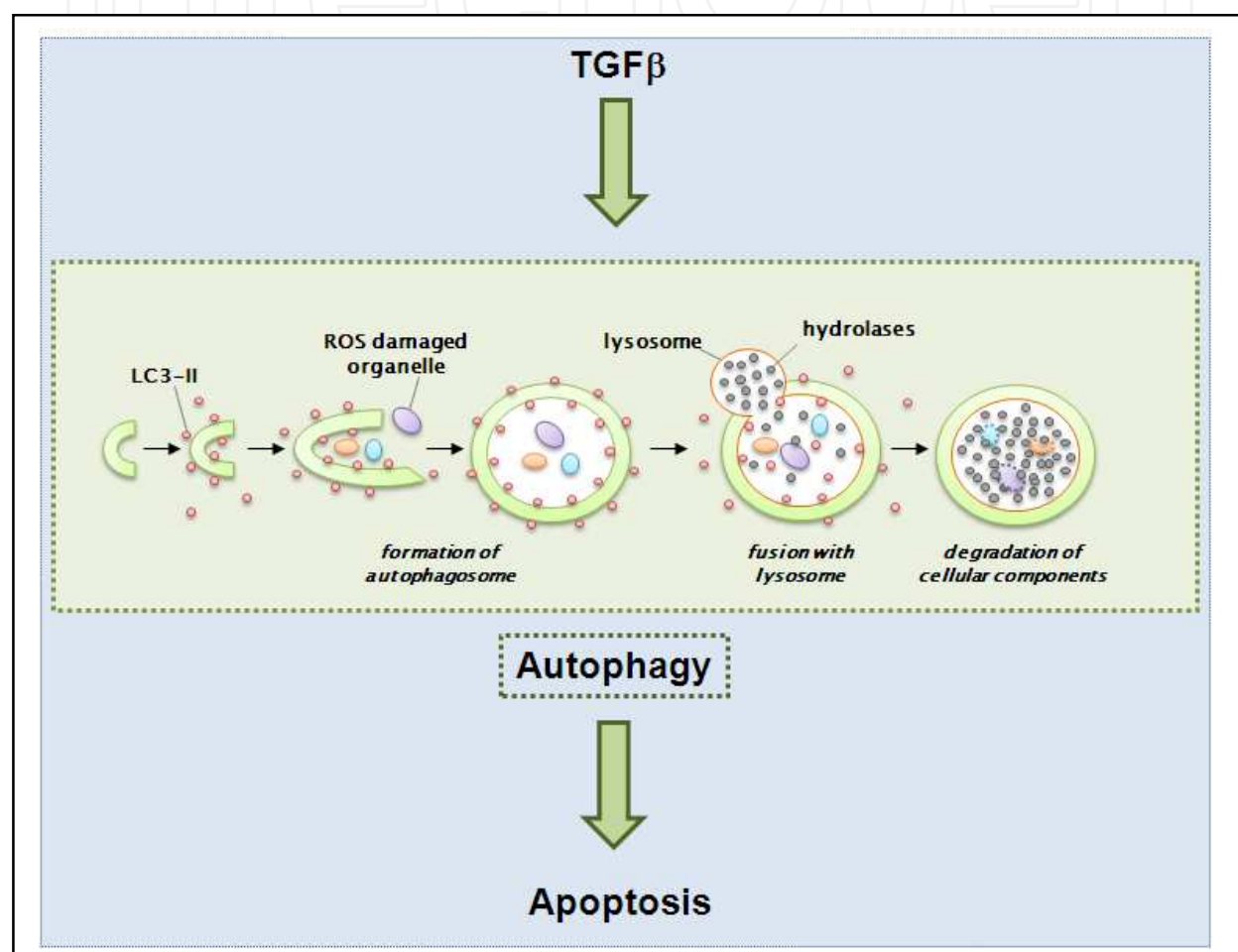
Fig. 3. Activation of the TGFβ signaling pathway induces autophagy. TGFβ can induce autophagosome formation in cancer cell lines via enhanced expression of autophagic markers (i.e. beclin-1, ATG5, and ATG7) and enhanced conversion of LC3-I to LC3-II. Induction of TGFβ -mediated autophagy occurs prior to apoptosis.

Supporting reports of TGFβ-induced autophagy arise from studies of renal epithelial cells which is involved in induction of peritubular fibrosis and degeneration of nephrons (Koesters et al., 2010). Opposing the concept that TGFβ leads to autophagic mediated cell death, TGFβ was reported to protect mesangial cells from apoptosis as a protective mechanism for survival during serum starvation via a TAK1 and AKT dependent pathway (Ding et al., 2010).

Our recent work has shown that upon exposure to reactive oxygen generating conditions (i.e. arsenic trioxide (As_2O_3) which is used to treat patients with acute promyelocytic leukemia (APL)), SnoN protein levels increase which coincides with induction of autophagy in a beclin-1 independent manner (Smith et al., 2010). Other TGFβ signaling mediators were examined and As_2O_3 was found to reduce the protein expression of EVI1, TAK1, SMAD2/3, and TGFβRII while increasing SnoN (Smith et al., 2010). Knockdown of SnoN via siRNA markedly reduced autophagy with a corresponding increase in apoptosis (Smith et al., 2010). Thus, disruption of induction of autophagy may be a novel therapeutic strategy to re-establish or increase sensitivity to therapeutic agents.

7. Targeting the TGFβ signaling pathway for therapy

Strategies need to be carefully designed for successful treatment of ovarian cancer patients via inhibition of TGFβ signaling pathway due to the apparent bifunctionality of TGFβ signaling. In particular, TGFβ levels, TGFβ receptor expression, and tumor stage/progression need to be assessed. There are in essence three major groups of TGFβ signaling therapeutics: (1) ligand traps including monoclonal TGFβ neutralizing antibodies and soluble TGFβRI/RII; (2) antisense molecule mediated silencing strategies for targeting TGFβ ligands; and (3) small molecule inhibitors targeting TGFβRI/RII and downstream mediators (Chou et al., 2010; Iyer et al., 2005; Korpal & Kang, 2010; Nagaraj & Datta, 2010). Neutralizing antibodies are designed to disrupt the interactions between TGFβ ligands and their cell-surface receptors (Chou et al., 2010). Some of these include 2G7 and 1D11 monoclonal antibodies which hinder the activity of all three TGFβ ligands to reduce tumor growth and metastasis (Chou et al., 2010). GC1008 is yet another neutralizing antibody which entered a Phase I/II clinical trial for advanced malignant melanoma and renal cell carcinoma patients (Chou et al., 2010). Soluble ligand traps include soluble TGFβRII/III which hinder TGFβ interaction with its cognate cell surface receptors leading to inhibition of tumor growth and metastasis in athymic murine models (Chou et al., 2010). Antisense oligonucleotides are yet another route to block TGFβ signaling, specifically against TGFβ1 gene expression which reduced tumor survival and metastasis in mouse models (Chou et al., 2010). In particular, AP12008, an antisense molecule which targets TGFβ2, effectively targets pancreatic and melanoma cell lines; it entered a Phase IIb clinical trial for patients with high grade gliomas with successful outcomes (Chou et al., 2010). However, the effectiveness of these large molecule inhibitors has limitations including adequately targeting a solid tumor due to physical barriers (Chou et al., 2010). Thus, small molecule inhibitors may be more effective and have been developed to initially target TGFβRI kinase activity with specificity (i.e. SB0431542, SD-208, LY580276, etc.). These act as competitive inhibitors of the ATP binding site of TGFβRI kinase (Chou et al., 2010). In addition, there now exists a dual inhibitor of TGFβRI and TGFβRII (LY2109761) which hinders metastatic process effectively (Chou et al., 2010). Other strategies that are being developed include small molecule inhibitors to directly inhibit SMAD-specific pathways as opposed to the non-canonical pathways (Chou et al., 2010).

In addition to the above targeting strategies, epigenetic therapy may also be another valuable therapeutic strategy for ovarian cancers with respect to targeting TGFβ transcriptional co-regulators such as EVI1. This strategy could potentially alter the epigenetic status leading to restoration of the expression of tumor suppressor genes with a

corresponding reduction in the expression of genes involved in mestastasis. For example, ADAM19, FBXO32, and RunX1T1 (tumor suppressors) are reduced in ovarian cancers but are normally increased in response to TGFβ; these genes are epigenetically silenced by promoter hypermethylation or histone modification (Balch et al., 2009).

With respect to SnoN/SkiL (Figure 4), based on our results with As_2O_3 in ovarian carcinoma cells, targeting of this TGFβ transcriptional co-regulator in ovarian cancers may lead to increased sensitivity to various chemotherapeutic agents.
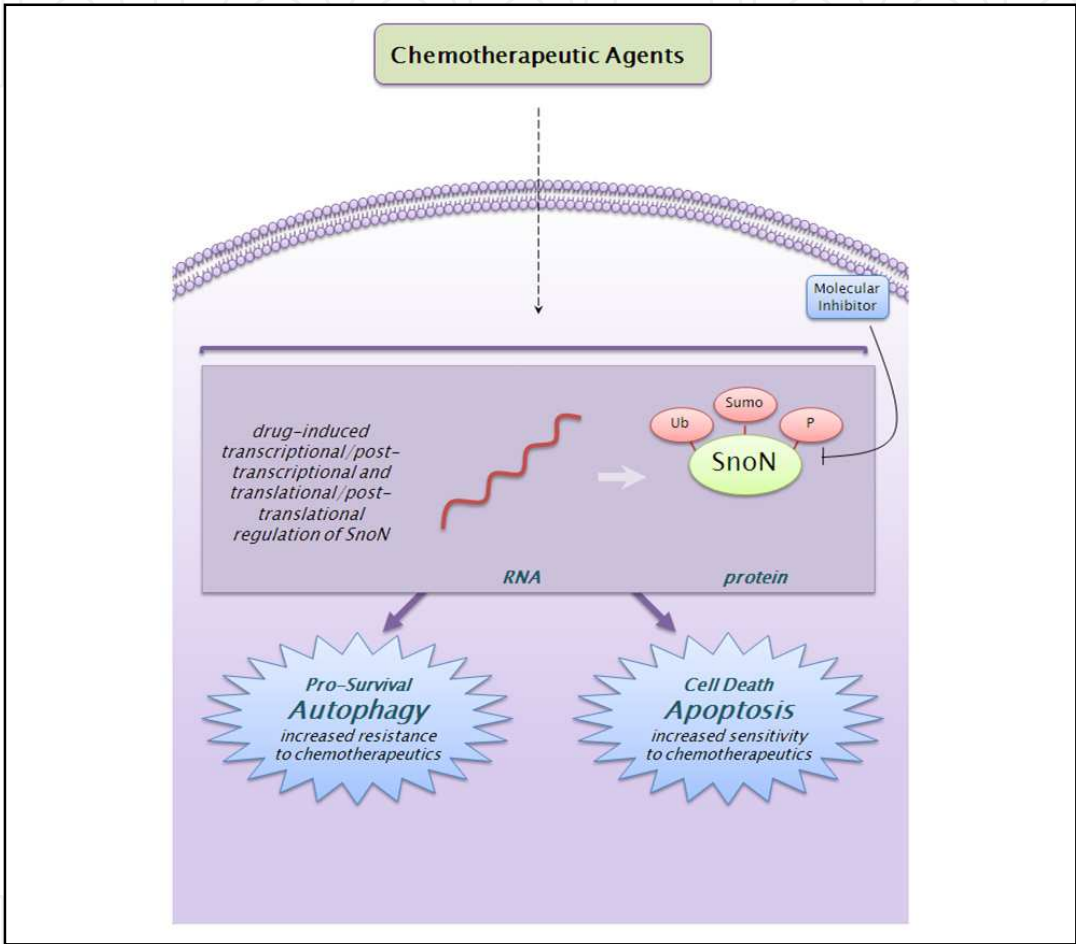
Fig. 4. Targeting of SnoN/SkiL to increase the sensitivity of ovarian cancer cells to chemotherapeutics. SnoN/SkiL levels increase following As_2O_3 treatment leading to induction of autophagy and increased resistance to the agent. Targeting of SnoN/SkiL with specific inhibitors may be a strategy to improve the sensitivity of the chemotherapeutic agents in ovarian cancer patients.

8. Conclusion

Although significant progress has been made in improving our understanding of the TGFβ signalling pathway, there remain numerous areas for further investigation to improve our understanding of the regulation of the TGFβ pathway. Thus, future research could possibly lead to development of novel and improved strategies for treatment of ovarian cancer patients.

9. Acknowledgements

10. References

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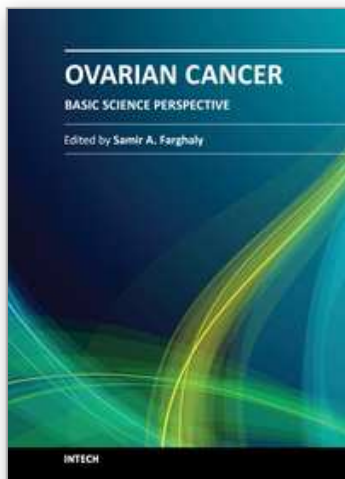
Ovarian Cancer - Basic Science Perspective

Edited by Dr. Samir Farghaly

Worldwide, Ovarian carcinoma continues to be responsible for more deaths than all other gynecologic malignancies combined. International leaders in the field address the critical biologic and basic science issues relevant to the disease. The book details the molecular biological aspects of ovarian cancer. It provides molecular biology techniques of understanding this cancer. The techniques are designed to determine tumor genetics, expression, and protein function, and to elucidate the genetic mechanisms by which gene and immunotherapies may be perfected. It provides an analysis of current research into aspects of malignant transformation, growth control, and metastasis. A comprehensive spectrum of topics is covered providing up to date information on scientific discoveries and management considerations.

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