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MOAP-1, UBR5 and cisplatin resistance in ovarian cancer

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Over 22,000 American women are diagnosed with ovarian cancer each year and 14,000 die of this disease, making it the fifth leading cause of cancer deaths among women and the number one gynecological cancer. Ovarian cancer is primarily treated with surgical debulking and chemotherapy that includes a taxane, such as paclitaxel or docetaxel, which interferes with the breakdown of the mitotic spindle during mitosis, and a platinum-based DNA-damaging agent, such as cisplatin or carboplatin. Due to relatively facile peritoneal dissemination of ovarian cancer cells, chemotherapy is of major importance in eliminating peritoneal micrometastases. Most patients with serous ovarian cancer initially respond to treatment, causing the disease to enter remission, but tumors often develop resistance to chemotherapy upon subsequent recurrences. Determining the mechanisms of platinum and taxol resistance is important to understanding disease progression and identifying novel therapeutic targets and strategies. Platinum resistance occurs through many mechanisms, including upregulation of DNA repair mechanisms, enhanced drug efflux from cells and downregulation of the cellular response to apoptosis, among others (1).

In a recent paper from Sally Kornbluth's lab, Matsuura *et al*. have discovered a novel mechanism of cisplatin resistance in ovarian cancer that works through destabilization of MOAP-1 (modulator of apoptosis 1), a protein with a BH3-like motif that stimulates activation of the pro-apoptotic Bcl-2 family protein Bax (2,3). MOAP-1 is an effector of the RASSF1A (Ras association domain family 1A) tumor suppressor that regulates extrinsic apoptosis through the TNFα (tumor necrosis factor α) and TRAIL (TNF-related apoptosis inducing ligand) pathways (4). Additionally, MOAP-1 can stimulate Bax activation in response to initiation of intrinsic apoptosis (2). Inactive MOAP-1 in non-apoptotic cells maintains a closed structure through an electrostatic, intramolecular interaction (5). Upon activation of an apoptotic signal, autoinhibition is disrupted and MOAP-1 uses its BH3-like domain to interact with Bax, stimulating Bax insertion into the mitochondrial membrane. This causes the release of cytochrome c and SMAC/Diablo from the mitochondria, leading to activation of downstream caspases and induction of apoptosis (2,4–6). Loss of MOAP-1 expression is, therefore, a possible means by which cancer cells could evade apoptotic cell

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death and, correspondingly, MOAP-1 expression is downregulated in some cancers (7). MOAP-1 protein has a half-life of only 25 minutes, suggesting strict regulation of protein turnover (8). Kornbluth's lab previously showed that MOAP-1 was a target for ubiquitination and degradation by the anaphase promoting complex [APC/C(Cdh1)], a process that is inhibited by the RING domain-containing protein Trim39 (9). APC/C(Cdh1) was shown to ubiquitinate MOAP-1 primarily in the G1 phase of the cell cycle, possibly allowing other ubiquitin ligases to act on MOAP-1 in other cell cycle phases.

Matsuura *et al*. now show that in S and G2 phases, MOAP-1 is targeted for degradation by the HECT (Homologous to the E6-AP Carboxyl Terminus) domain-containing E3 ubiquitin ligase UBR5 (Ubiquitin Protein Ligase E3 Component N-Recognin 5) (3). UBR5 is a 309 kDa nuclear phosphoprotein that has reported roles in the DNA damage response and G2 checkpoint control (10–12). UBR5 overexpression in tumors could help tumor cells evade the cytotoxic effects of DNA damaging agents by influencing DNA repair, cellular checkpoints and the apoptotic response to cell injury. UBR5 is upregulated in several cancer types and its upregulation in ovarian cancer is associated with a doubled risk of recurrence and death (13,14). UBR5 is also upregulated in several ovarian cancer cell lines (15). Kornbluth's lab identified UBR5 as a MOAP-1 binding partner in a co-immunoprecipitation after etoposide treatment and speculated that it might regulate MOAP-1 ubiquitination and degradation (3). Indeed, they showed that UBR5 knockdown prolonged MOAP-1 protein half-life and decreased MOAP-1 poly-ubiquitination in cells. Moreover, UBR5 directly polyubiquitinated MOAP-1 *in vitro*. UBR5 is a part of the EDVP E3 ubiquitin ligase complex, which is composed of UBR5, DDB1, VPRBP and the protein kinase DYRK2, with some substrates being targeted for phosphorylation by DYRK2 prior to ubiquitination by UBR5 (16). While it is unknown if MOAP-1 is a DYRK2 substrate, MOAP-1 associated with each component of the complex in co-immunoprecipitations, the addition of purified components of the complex enhanced MOAP-1 ubiquitination *in vitro* and DYRK2 knockdown enhanced MOAP-1 half-life in cells (3). Interestingly, UBR5 specifically regulated MOAP-1 protein levels in a cell cycle-dependent manner. Cell synchronization via release from a double thymidine block, combined with UBR5 knockdown, demonstrated that UBR5 downregulation enhanced MOAP-1 protein levels starting in S phase and this continued through the following early G1 phase, based on monitoring cyclin B levels. Previous studies suggested that UBR5 mainly works at this latter stage of the cell cycle (11,17). This raises the question of how the interaction between UBR5 and MOAP-1 is regulated. Through all of the cell cycle except mitosis the nuclear envelope is intact. UBR5 contains two nuclear localization sequences (NLSs) and appears to localize exclusively in the nucleus, even upon ectopic overexpression (10,15). MOAP-1 has mainly been characterized for its localization to the mitochondrial membrane through its N-terminal domain and its localization with RASSF1A and death receptors at the plasma membrane through its C-terminal domain (2,18,19). However, at 39.5 kDa, MOAP-1 is small enough for passive diffusion through the nuclear pore. Its KRRR sequence, a potential NLS, is involved in the intramolecular electrostatic interaction and upon release of autoinhibition, MOAP-1 binds to Bax (5). In the closely-related protein PNMA5, which does localize to the nucleus, this identical sequence does not confer nuclear localization (20). Additionally, an EGFP-MOAP-1 fusion protein, which is too large for passive diffusion to the nucleus,

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localized to the cytoplasm when overexpressed alone and to microtubules in the presence of exogenous RASSF1A (4). Further study of changes in MOAP-1 localization and potential modification by DYRK2 is needed in order to understand the mechanism by which the UBR5/MOAP-1 interaction occurs.

UBR5 is known to modulate the cellular response to cisplatin through its ubiquitin ligase activity. Our lab demonstrated that UBR5 downregulation with siRNA or shRNA enhances cisplatin sensitivity in ovarian cancer cells *in vitro*, as shown by changes in dose toxicity curves and increases in PARP (poly ADP ribose polymerase) cleavage, while O'Brien *et al*. showed that UBR5 knockdown sensitized cells to cisplatin in a colony forming assay (13,15). Matsuura *et al*. confirmed the sensitization to cisplatin upon UBR5 knockdown and showed that co-knockdown of MOAP-1 reduced the cisplatin sensitization effect of UBR5 knockdown, suggesting that UBR5 uses MOAP-1 in part to induce cisplatin resistance (3). Moreover, our lab showed that ectopic overexpression of UBR5, but not a UBR5 point mutant in which the critical cysteine required for its ubiquitin ligase activity was mutated, enhanced cisplatin resistance in cells (15), showing that overexpression of UBR5 alone was sufficient to induce cisplatin resistance, dependent upon its ability to ubiquitinate its substrates. This appears to reflect the situation in human tumors, as Matsuura *et al*. showed that UBR5 and DYRK2 are upregulated in tumor samples from ovarian cancer patients who had a poorer response to initial platinum-based chemotherapy compared to tumors from those patients with a better initial response to treatment (3). By identifying MOAP-1 as a novel UBR5 substrate that is involved in cisplatin resistance, they have identified one potential mechanism by which UBR5 may regulate the cellular and tumor response to cisplatin. However, there was not a clear down-regulation of MOAP-1 protein in the patient samples that correlated with poorer cisplatin response, suggesting that other UBR5 substrates are likely involved in the induction of cisplatin resistance (3). Like protein kinases, ubiquitin ligases can have many substrates that they modify and they may need to modify several to bring about a biological response. Modification of MOAP-1 levels may be one component of this process in tumors or may be the mechanism of UBR5-induced cisplatin resistance in a subset of ovarian tumors. Identification of additional UBR5 substrates is needed to understand the complete mechanism of UBR5-induced cisplatin resistance and to identify other functions of UBR5.

This is not the only instance of UBR5 regulating the expression of a protein involved in the apoptotic machinery. The central third of the UBR5 protein can act as a transcriptional coactivator (10). We showed that UBR5 downregulation resulted in loss of endogenous, but not exogenous, Mcl-1 protein and mRNA, and that UBR5 transfection upregulated an Mcl-1 promoter-driven luciferase construct, independent of UBR5 ubiquitin ligase activity. Thus, UBR5 may impact apoptosis through both ubiquitin ligase-dependent and -independent mechanisms, acting on Bcl-2 family proteins and their regulators.

As noted, new therapies for ovarian cancer treatment are required for enhancing patient outcomes. Matsuura *et al*. conclude, as we did, that UBR5 may be a potential therapeutic target for epithelial ovarian cancer to enhance the efficacy of platinum-based drugs. Indeed, we showed that nanoparticle delivery of UBR5 siRNA to established ovarian cancer xenografts in mice enhanced cisplatin efficacy (15). Developing small molecule inhibitors to

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E3 ligases has generally proven to be difficult, much more so than for protein kinases, although there have been some successes. Efforts to identify clinically useful inhibitors of UBR5 could enhance platinum-based therapy in ovarian cancer patients.

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