Editorial

Mechanisms Underlying Chemoprevention of Ovarian Cancer

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Ovarian cancer remains the most lethal of the gynecological cancers. Although relatively uncommon, afflicting ~1 of 70 women in the United States, the high mortality rate makes this disease a major health concern. The high mortality rate arises from the lack of an effective screening approach combined with inadequate therapeutic approaches for advanced disease. Indeed, fewer than 25% of ovarian cancers are identified at an early curable stage. Other than family and personal history of cancer or the inheritance of abnormalities in the BRCA1 or BRCA2 or other ovarian cancer predisposition genes, there are few indicators that a women has an elevated risk of development of cancer. Thus, it is difficult to identify women warranting prophylactic surgery or intensive screening. The high mortality rate combined with the lack of effective screening approaches make ovarian cancer a strong candidate for chemoprevention (1).

In this issue of "Clinical Cancer Research," Rodriguez-Burford *et al.* (2) explore the potential mechanisms underlying epidemiological observations that ovarian cancer occurs at a lower frequency in women exposed to NSAIDS.[2] Cramer *et al.* (3) in a population-based, case-control study demonstrated that a 6-month exposure to acetaminophen at least 1 day/week, but not aspirin or ibuprofen, resulted in a statistically significant 2-fold decrease in the lifetime risk of developing ovarian cancer. Moysich *et al.* (4) in a hospital-based, case-control study also demonstrated a time- and dose-dependent association of acetaminophen, but not aspirin use, with decreased risk of developing ovarian cancer. Rosenberg *et al.* (5) in a large-scale, case-control study failed to confirm the association of moderate use of acetaminophen and decreased risk of ovarian cancer demonstrated by Cramer *et al.* (3) but did identify a statistically significant decrease in development of ovarian cancer with prolonged acetaminophen use, at least ≥4 days/week for 5 years. Tavani *et al.* (6) has failed recently to detect an association of aspirin use with prevention of ovarian cancer. In contrast to these reports, Akhmedkhanov *et al.* (7) demonstrated a 2~3-fold decrease in epithelial ovarian cancer associated with aspirin use. Taken together, although controversial, these epidemiological observations suggest that an improved understanding of the mechanism(s) by which NSAIDS may decrease the development of ovarian cancer could lead to improved approaches for chemoprevention of this deadly disease.

The studies in this issue of "Clinical Cancer Research" identify several of the problems associated with attempting to use cell models to understand the mechanisms underlying epidemiological studies of chemoprevention of epithelial ovarian cancer. At this point, there is no readily manipulatable mammalian model of epithelial ovarian cancer. Indeed, the only extant animal model with reproducible epithelial ovarian cancer is a chicken model (8). This model, while contributing to our understanding of chemoprevention of ovarian cancer, is far from optimal. The mouse models of human cancer consortium, as well as members of the ovarian cancer research community, are investigating a number of approaches to develop practical animal models; however, at the present, it is necessary to rely on ovarian cancer cell lines, cultures of normal ovarian epithelium, or normal epithelium with enforced expression of genes, such as the large T antigen of SV40, telomerase, and oncogenes to attempt to explore the mechanisms underlying chemoprevention of epithelial ovarian cancer. These established cell culture systems and, in particular, ovarian cancer cell lines may not adequately reflect the transformation processes targeted by chemoprevention agents *in vivo*.

The studies by Rodriguez-Burford *et al.* (2) demonstrate that acetylsalicylic acid, acetaminophen, and a Cox-2 inhibitor, NS-398, can decrease the growth of fully transformed human epithelial ovarian cancer cells. The Cox-2 agent both decreased cell proliferation in established cell lines and induced apoptosis in freshly isolated ovarian cancer cells. This information implicates cellular proliferation and survival in the action of NSAIDS on the development of epithelial ovarian cancer. Although all three NSAIDS decreased the proliferation of ovarian cancer cell lines, concentrations far above the therapeutic range and well above the maximal tolerated dose and, in particular, the chemopreventative doses of the inhibitors were required to demonstrate antiproliferative effects *in vitro* (2). This is compatible with the observation that in contrast to bowel cancer cells, which are very sensitive to the effects of NSAIDS likely as a consequence of overexpression of Cox2 (9–12) and dependent on Cox2 function for growth and survival, human epithelial ovarian cancer cells appear to express Cox1 and Cox2 at low levels (2, 13). Thus, either a prolonged low-dose exposure to the NSAIDS has effects on Cox2 function in normal or partially transformed ovarian epithelium, or NSAIDS exert their effects through alternative mechanisms.

The observation that very high concentrations of NSAIDS are required to decrease the proliferation of ovarian cancer cell lines and that ovarian cancer cells express low levels of COX1 and COX2 (2, 13) suggests that the effects of NSAIDS on ovarian cancer prevention may be attributable to mechanisms independent of inhibition of COX1 and COX2 as reflected by the effects of NSAIDS on the proliferation or survival of established ovarian cancer cell lines. In this aspect, it is important to note that the epidemiological data most strongly implicate acetaminophen as compared with aspirin or ibuprofen in the prevention of ovarian cancer (2–7). Acetaminophen is a relatively poor inhibitor of cyclooxygenase and does not have

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[2] The abbreviations used are: NSAID, nonsteroidal anti-inflammatory agent; PI3K, phosphatidylinositol 3′-kinase; LPA, lysophosphatidic acid.

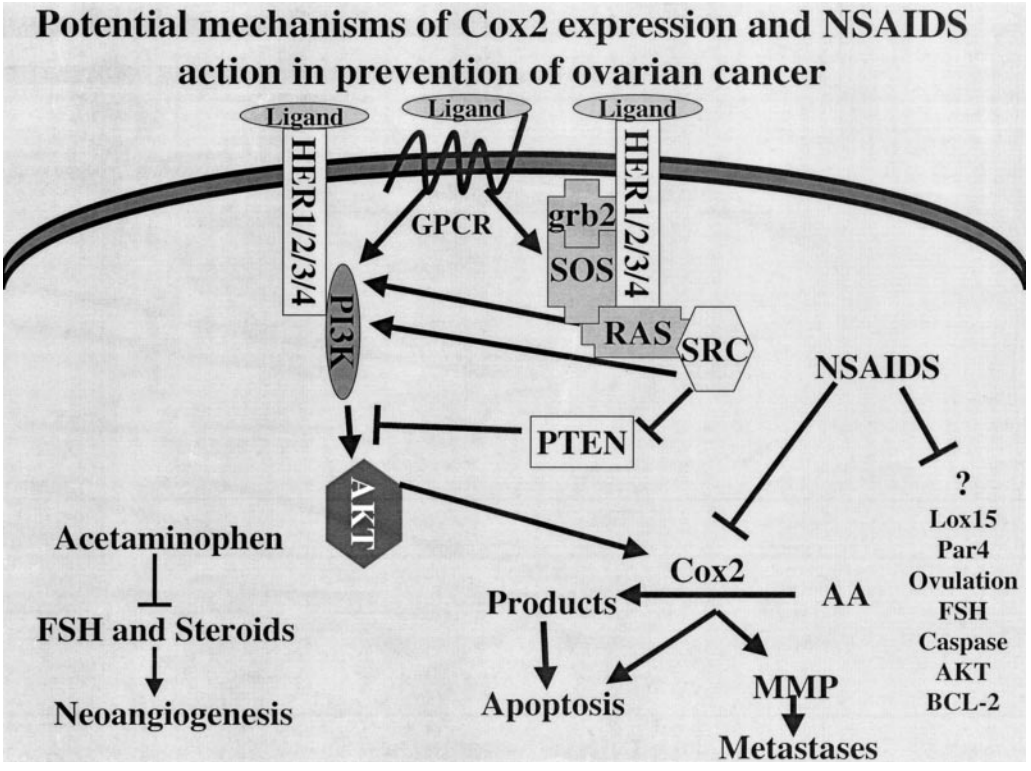

Fig. 1 Potential mechanism of Cox2 expression and NSAID action in the prevention of ovarian cancer. AKT has been demonstrated to be a potent regulator of Cox2 expression and action. Multiple mechanisms result in constitutive activation in ovarian cancer cells. Ovarian cancer cells can overexpress members of the human epidermal growth factor receptor kinase family (*HER*). The HER family efficiently couples to both the *PI3K* and RAS pathways, as well as to the SRC proto-oncogene. In addition, the LPA receptors 2 and 3 of the G protein-coupled receptor family are expressed at elevated levels in ovarian cancer cells. In ovarian cancer cells, these receptors couple efficiently to PI3K, RAS, and SRC. In the presence of LPA, which is itself increased in ovarian cancer, the LPA Multiple components of the PI3K pathway are amplified in ovarian cancer as a consequence of increased DNA copy number, including the PI3K catalytic subunit and AKT1 and 2. In addition, PTEN, which functions as a tumor suppressor gene to inhibit activation of AKT by PI3K, is expressed at decreased levels and can be mutated, particularly in endometrioid ovarian cancers. This renders the PI3K pathway hyper-responsive to ligand in ovarian cancer cells. The RAS signaling pathway also impinges on AKT activity by activating PI3K. SRC, which is increased in amount and activity in ovarian cancer cells, impinges on the PI3K AKT pathway by increasing PI3K activity, as well as by phosphorylating and decreasing PTEN activity. NSAIDS inhibit Cox2, decreasing both production of matrix metalloproteinases and apoptosis. An ability to invade and bypass apoptosis is required for activation of the metastatic cascade. NSAIDS either through Cox2 or through as-yet unknown mechanisms can inhibit Lox15, Par4, ovulation, follicle-stimulating hormone production, activation of caspase proapoptotic mediators, AKT activation, and function of the BCL-2 antiapoptotic mediator. In addition to these effects, acetaminophen, which is a relatively weak Cox2 inhibitor, may decrease the production of ovarian steroids and follicle-stimulating hormone, which can decrease ovulation and alter neoangiogenesis, respectively, thus decreasing tumor development.

significant chemopreventative activity against bowel cancer (2, 3). Given that other NSAIDS and particularly Cox2 inhibitors appear to demonstrate chemopreventative activity against bowel cancer (9–12), it seems likely that acetaminophen is exerting its effects through a non-Cox2-dependent mechanism.

The development of ovarian cancer has been strongly linked to the frequency of ovulation and also to the high concentrations of gonadotropins present postmenopausally (14–21). The association with ovulation has been linked to the proliferation of the ovarian epithelium, and potentially, the entrapment of ovarian surface epithelium cells within the stroma associated with repair of the ovulatory defect. Gonadotropins, in contrast, have been demonstrated to regulate neoangiogenesis in ovarian cancer models, a critical component of the development and metastases of tumors in many lineages.

The first and strongest epidemiological evidence demonstrating that chemoprevention was effective at inhibiting the development of any major life-threatening cancer was derived from studies of ovarian cancer. Prolonged (5 years) use of the oral contraceptive pill significantly reduces the risk of developing ovarian cancer (odds ratio, 0.25–0.8; Refs. 22–27). Strikingly, decreased risk can be observed for at least 10 years after discontinuation of oral contraceptives. Although there are multiple potential causes, the most likely mechanism by which oral contraceptives prevent ovarian cancer is through decreasing the frequency of ovulation and, thus, the mutagenic effects of cellular proliferation and entrapment of the ovarian epithelium within the stroma.

The epidemiological evidence linking ovulation and gonadotropin levels to development of ovarian cancer suggests alternative mechanisms by which NSAIDS and, in particular, acetaminophen could impact the development of ovarian cancer (Fig. 1). Acetaminophen contains structural moieties

reminiscent of steroidal hormones potentially contributing to its ability, at high doses, to induce uterine, ovarian, and testicular atrophy in rats (2, 3, 28). This suggests that acetaminophen may decrease ovulation. In support of this contention, several studies have indicated that NSAIDS can decrease productive ovulation in animal and human models (29–32). Thus, acetaminophen could decrease the frequency of ovulation and the "wear-and-tear" damage on the at-risk tissue, the ovarian epithelium, that contributes to the accumulation of mutations in the epithelium and the development of ovarian cancer. Acetaminophen also alters glutathione metabolism potentially leading to alterations in gonadotropin levels (33). Thus, NSAIDS may exhibit activities on ovarian epithelium through multiple mechanisms unrelated to proliferation and survival.

Ovarian cancer, like all cancers, occurs as a consequence of either germ-line or acquired somatic changes in genetic function (14, 34–36). The required alterations in gene expression or function can occur as a result of mutations or of epigenetic alterations, such as changes in methylation (14, 34–36). One important challenge is to link the genotypic changes that occur in ovarian cancer cells to the phenotypic and biological changes observed in human tumors. Linking epidemiology, mechanistic studies of potential chemoprevention agents, and studies of the underlying genetic events leading to the development of ovarian cancer is critical for our understanding of the pathophysiology of ovarian cancer. Development of practical animal models to test hypotheses generated through genetic and epidemiological studies of human ovarian cancer should greatly facilitate the development of effective chemopreventative approaches for this devastating disease.

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