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Signal transduction based drug development for ovarian hyper-stimulation and anti-cancer treatment

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Regulation of the gonadotropic response through modulation of EGF-family proteins expression

Gonadotropins play a crucial role in ovarian homeostasis and fertilization through the activation of the cAMP cascade. However, gonadotropin hyper-stimulation may be associated with elevated risk for ovarian cancer. It has been suggested, that high gonadotropin levels in peritoneal and ovarian cystic fluids of patients may lead to malignancy. Moreover, we have recently discovered that gonadotropin stimulation can activate the MAPK cascade in target cells. Using DNA microarray technology and RNA from human granulosa cells, we discovered that stimulation with saturating doses of gonadotropins dramatically elevates activity of genes coding for epiregulin and amphiregulin. These gene products can bind and activate the EGF receptor and ERBB4, which are associated with the development of ovarian, breast endometrial and other non-gynecological cancers. Since gonadotropin receptors are expressed not only in the gonads, but also in non-gonadal tissues and cancer cells, blocking the expression of epiregulin and amphiregulin, which belong to the EGF family, in cancer tissue may attenuate or arrest cancer cells proliferation. This could be achieved using anti-sense and/ or SiRNA techniques targeted to suppress the expression of the gonadotropin receptors and/ or the growth factors of the EGF family. Thus, the discovery that gonadotropins activate certain mitogenic signal transduction pathways, may serve as a guide for the treatment of specific cancers by blocking the gonadotropic response, either on the receptor level or downstream on mitogenic signals generated by these hormones. Moreover, we and others recently demonstrated that amphiregulin and epiregulin act as mediators of luteinizing hormone (LH) action in the mammalian ovulatory follicles and in isolated ovarian follicular granulosa cells. Therefore, regulation of the expression of these factors may open new possibilities in treatment of ovarian malfunction implicated with ovarian hyper-stimulation.

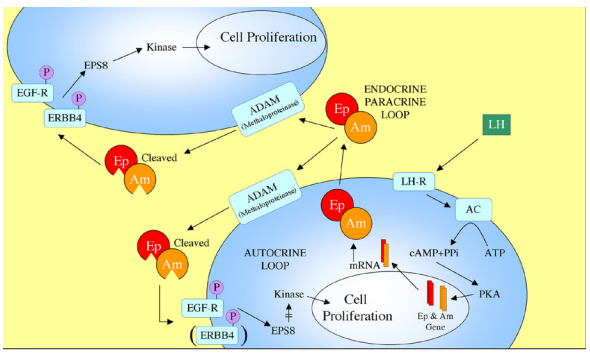

Fig. 1 Tentative LH involvement in ovarian cancer development implicated in epiregulin and amphiregulin expression. *LH mediates Epiregulin (Ep) and amphiregulin (Am) synthesis through cAMP accumulation. When activated, by metaloproteinases (ADAMs), Ep and Am bind EGF receptor (EGF-R) or ERBB4, thus activating a mitogenic pathway in adjuscent cells, or distal cells and tissues. An autocrine loop doesn't seem to exist in normal granulosa cells, where ADAMs, ERBB4 and epidermal growth factor receptor substrate 8 (EPS8) expression is down regulated.*

Phosphodiesterase inhibitors as anti-cancer drugs

It is well known that high intracellular levels of cAMP can effectively kill cancer cells *in vitro*. Unfortunately substances elevating cAMP such as forskolin, 8-bromo-cAMP, 8-chloro-cAMP, mono- or dibutiryl cAMP are not recommended to be used as anti- cancer drugs, because of their high cytotoxicity. In contrast blockers of phosphodieterases such as theophylline and aminophylline, which could elevate intracellular cAMP, are commonly used as anti-asthma drugs reaching concentrations in the

blood of 20-25μg/ml. We tested the effectiveness of theophylline and aminophylline to induce cell death alone or in combination with common anti-cancer drugs such as cisplatin and gemcitabine (gemzar). We examined such drug combinations in the induction of cell death in a variety of carcinoma cell lines derived from human ovarian, prostate and lung cancer. While theophylline could induce moderate cell death alone, at 20-25 μg/ml concentrations, aminophylline was ineffective at this concentration. Theophylline was found in all three representative cell lines to synergize with gemcitabine and cisplatin to induce programmed cell death, which permits a reduction in the effective doses of cisplatin and gemcitabine by 2-3 fold. The effect of theophylline in induction of apoptosis involved reduction of intracellular levels of Bcl-2. Such a reduction was proportional to the extent of apoptosis induced by theophylline as well as by the combined drug treatments. Therefore, we propose that theophylline should be considered as a potential anti-cancer drug in combination with other chemotherapeutic drugs. Screening of other phosphodiesterase blockers, which are not severely toxic, could open a possibility to improved chemotherapeutic cancer treatments with reduced undesired side-effects. A clinical trial, using theophylline as an anti-cancer drug, is currently being conducted in lung cancer patients.

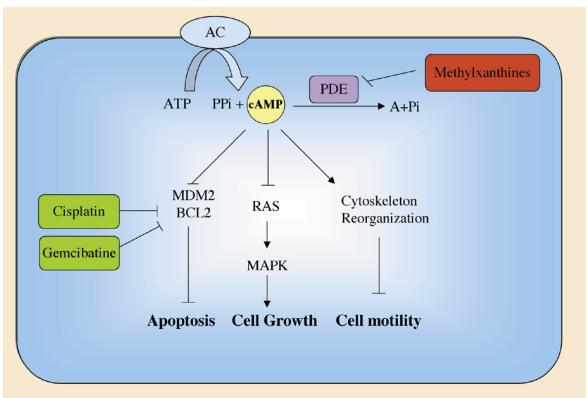

Fig. 2 Possible action of methylxantines as anti-cancer drug. *Methylxantines like Theophylline block phosphodiesterase (PDE), thus elevating cyclic AMP (cAMP) level. This may down-regulate the anti-apoptotic proteins MDM2 and BCL2. Theophylline, cisplatin and/or gemcitabine may synergize in down-regulating survival protein, thus stimulating apoptosis in the cancer cells. Elevated cAMP levels may also down-regulate RAS/MAPK signaling, in a cell specific manner, leading to arrest in cell growth and block cell motility by cytoskeletal reorganization.cells, where ADAMs, ERBB4 and epidermal growth factor receptor substrate 8 (EPS8) expression is down regulated.*

Selected Publications

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