A Role for cAMP-Driven Transactivation of EGFR in Cancer Aggressiveness
– Therapeutic Implications

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Abstract

In many common cancers, production of cAMP boosts cancer proliferation, survival, and aggressiveness, reflecting the fact that, through mechanisms that require further clarification, cAMP can promote tyrosine phosphorylation, notably transactivation of the epidermal growth factor receptor (EGFR). Hormones which activate adenylate cyclase in many cancers include PGE2 – often produced by cox-2 activity within tumors – and adrenergic hormones, acting on beta2 receptors. NSAID cyclooxygenase inhibitors, including low-dose aspirin, clearly reduce risk for many adenocarcinomas, but the impact of cox-2 inhibitors in clinical cancer therapy remains somewhat equivocal. There is increasing evidence that increased sympathetic drive, often reflecting psychic stress or tobacco usage, increases risk for, and promotes the aggressiveness of, many cancers. The non-specific beta antagonist propranolol shows cancer-retardant activity in pre-clinical rodent studies, especially in stressed animals, and a limited amount of epidemiology concludes that concurrent propranolol usage is associated with superior prognosis in breast cancer and melanoma. Epidemiology correlating increased resting heart rate with increased total cancer mortality can be interpreted as compelling evidence that increased sympathetic drive encourages the onset and progression of common cancers. Conversely, hormones which inhibit adenylate cyclase activity in cancers may have potential for cancer control; GABA, which can be administered as a well-tolerated nutraceutical, has potential in this regard. Combination regimens intended to down-regulate cancer cAMP levels, perhaps used in conjunction with EGFR inhibitors, may have considerable potential for suppressing the contribution of cAMP/EGFR to cancer aggressiveness.

cAMP as a Mediator of Cancer Agressiveness

There is growing evidence that hormones which boost cAMP production play an important role in the onset and spread of many common cancers. This reflects the fact that, in many tissues, cAMP can somehow promote the tyrosine phosphorylation of protein platforms. In particular, a number of studies demonstrate that cAMP/PKA activity, and hormones that stimulate Goα-coupled receptors that trigger such activity, have the potential to transactivate the epidermal growth factor receptor (EGFR), thereby promoting the many signals triggered by tyrosine phosphorylation.1-23 This phenomenon appears to be enhanced by cellular transformation,17 and its downstream consequences, in concert with other effects of cAMP24 and of PKA activity, can contribute importantly to the proliferation, survival, and aggressive spread of cancers. In particular, in many cell types this can lead to activation of PI3K/Akt, Ras-Erk1/2, AP-1, Stat3, NF-kappaB, and CREB, and increased expression of VEGF, IL-6, IL-8, and metalloproteinases25-42 - signals and proteases that are constitutively active drivers of malignant behavior in many cancers. The mechanism by which PKA induces EGFR transactivation appears to vary by cell type and has not been clearly defined; a mediated factor is activation of the tyrosine kinase c-Src, which can either promote tyrosine phosphorylation of EGFR via an intracellular pathway, or by activating matrix metalloproteinase activities that release EGFR ligands such as TGF-alpha. PKA may activate c-
Src by phosphorylating Ser-17, which disinhibits an autophosphorylation of Tyr-419 that locks c-Src into an activated conformation.\textsuperscript{43}

The ability of PKA to promote transactivation of EGFR somewhat preferentially in transformed cells seems likely to reflect the fact that the RI regulatory subunit of PKA tends to be expressed constitutively in cancer cells, whereas in healthy cells it is expressed only transiently in response to proliferative signals.\textsuperscript{44-48} PKA is a tetramer composed of two identical catalytic subunits and two identical regulatory subunits; the binding of cAMP to the regulatory subunits causes the tetramer to break apart, releasing the catalytic subunits and enabling their kinase activity. Whereas there is only one form of the catalytic subunit, there are two isoforms of the regulatory subunit, RI and RII, and hence there are two subtypes of PKA. The regulatory subunits modulate PKA activity by determining its subcellular location; they also have differing affinities for cAMP. RI can bind to Grb2, which implies that it can associate with certain tyrosine-phosphorylated growth factor receptors such as EGFR.\textsuperscript{44,49} Abrogation of RI expression in cancer cell lines leads to up-regulation of the RII subunit, with the result that the cAMP/PKA signal tends to inhibit cell proliferation and promote differentiation; hence, PKA activity can either prevent or promote malignant behavior, dependent on the relative expressions of its R1 and RII regulatory subunits.\textsuperscript{48} In healthy cells, cAMP can either up-regulate or down-regulate cellular proliferation and MAP kinase signaling,\textsuperscript{50} dependent on the cell type and its differentiation state. It should be noted that in some types of cancer, such as gliomas, lymphomas and leukemias, cAMP can exert a cancer-retardant effect.\textsuperscript{51-53} Nonetheless, this should not obscure the fact that in the large majority of common cancers, cAMP has a pro-proliferative impact.

These findings suggest that feasible measures which suppress cAMP generation in cancers, in conjunction with drugs that blunt the activation of EGFR, may often be useful for cancer control; indeed, several research groups have presented evidence that use of EGFR inhibitors (such as the clinically approved erlotinib) in concert with measures that lower cAMP in cancer cells, can be markedly effective for slowing the growth of certain cancers, and sensitizing them to cytotoxic agents or radiation.\textsuperscript{54-60} Potentially, measures which suppress RI expression – such as the antisense oligonucleotide GEM231, which has shown anti-cancer activity in pre-clinical models\textsuperscript{61-63} – could also be employed to prevent PKA-mediated transactivation of EGFR.

**Cox-2 and PGE2 as Determinants of Cancer Risk**

The hormonal signals which stimulate cAMP production in cancers evidently vary according to cancer type, but two mechanisms in particular are common to many cancers – PGE2 activation of its receptors EP2/EP4, and epinephrine/norepinephrine activation of beta2 adrenergic receptors. The latter receptors are readily inhibited with broad-spectrum beta antagonists such as propranolol; and whereas drugs which inhibit specific PGE2 receptors are not yet clinically available, cyclooxygenase inhibitors (notably inhibitors of cox-2, overexpressed in many cancers) can be employed to inhibit PGE2 production.

Epidemiology suggests that cyclooxygenase inhibitors – NSAIDs – can reduce the incidence of many cancers, and a meta-analysis of controlled trials of daily low-dose aspirin administration demonstrates convincingly that even a modest but consistent level of cyclooxygenase inhibition can be effective in this regard.\textsuperscript{64-66} Cox-2-selective inhibitors have shown cancer-control utility in pre-clinical models, and have been employed as components of various effective clinical drug regimens for cancer, but controlled clinical trials evaluating their contribution to the efficacy of such regimens have often yielded null
Although low-dose aspirin has been found to decrease the tendency of many cancers to metastasize, this phenomenon might conceivably reflect platelet stabilization rather than inhibition of COX-2 in tumors. It remains unclear as to why cyclooxygenase inhibition appears to be more active for cancer prevention, than for control of existing cancers.

**Beta2 Adrenergic Activity Boosts cAMP in Many Cancers**

Beta2 adrenergic receptors are expressed by a high proportion of common cancers, and there is growing evidence that the negative impact of psychological stress or cigarette smoking on cancer prognosis may be mediated primarily by increased exposure of these receptors to epinephrine (secreted by the adrenal gland) and norepinephrine (from sympathetic nerve terminals). Psychological stressors, nicotine administration, or continual infusion of norepinephrine have been shown to accelerate the growth or spread of certain cancers in rodents, and this effect can be blocked with propranolol or other beta2 antagonists. Remarkably, nicotine can stimulate autocrine production of norepinephrine and epinephrine in certain cancer cell lines (pancreatic, gastric, and non-small-cell lung cancers), resulting in growth stimulation that is blocked by propranolol. Propanolol administration can increase the responsiveness of certain cancers to cytotoxic drugs or radiation, both in vitro and in vivo, and is effective as a single agent in treating angiosarcoma xenografts. Although epidemiological studies examining the impact of beta-blocker usage on human cancer incidence have not consistently confirmed a protective effect – possibly in part because many patients now use cardiospecific beta1 antagonists – the use of beta-blockers or of propranolol specifically has recently been linked to decreased metastatic spread and improved survival in patients with melanoma and breast cancer, and to reduced risk for hepatocellular carcinoma in high-risk patients. In a recent analysis of 1,425 patients with epithelial ovarian cancer, the 195 patients using non-selective beta blockers had a median overall survival of 7.91 years, as contrasted with 3.5 years for non-beta-blocker users, and 3.17 years in users of cardioselective beta-blockers.

Resting heart rate can be viewed as a rough correlate of systemic sympathetic activity. Over 30 years ago, an analysis by Stamler and colleagues observed that, after multivariate adjustments, resting heart rate correlated positively with total cancer mortality in two of three prospective data bases that were examined. At least three subsequent prospective studies have concluded that resting pulse rate correlates with increased risk for cancer mortality in men. Importantly, two of these studies found that this relationship persisted after adjustment for other established cancer risk factors including BMI, smoking, and physical activity. Obesity/metabolic syndrome, tobacco use, and lack of exercise can all raise the pulse rate while also increasing cancer risk and mortality; the fact that heart rate persists as a determinant of cancer mortality after statistical adjustment for these factors is consistent with the thesis that sympathetic drive per se increases risk for cancer death, and may in fact mediate, in part, the increased cancer risk associated with metabolic syndrome, smoking, and sedentary lifestyle. Prospective analyses examining prostate cancer specifically have concluded that heart rate correlates positively with prostate cancer incidence and mortality, albeit one study could not confirm such a relationship. Inasmuch as most of these studies deal with cancer mortality – as opposed to incidence – they do not clarify to what extent an impact on cancer prognosis may have played a role in the higher cancer mortality observed with high pulse rate.
Heart rate is determined by the balance between sympathetic and parasympathetic input to the heart. In the absence of any evidence suggesting that parasympathetic activity is a determinant of cancer risk, and in light of recent evidence that adrenergic activity can have a promotional impact on many cancers, it is logical to suspect that sympathetic drive is the mediator of the increased cancer mortality associated with increased heart rate. Urinary catecholamines also can be employed as a marker for systemic sympathetic activity. Women at high familial risk for breast cancer have been found to have higher urinary excretion of epinephrine during the work day.\textsuperscript{134} Perhaps this reflects a role for increased sympathoadrenal activity in the genesis of some breast cancers. There appear to be few if any other studies attempting to correlate urinary catecholamine levels with cancer risk or progression (excepting the special case of pheochromocytoma).

In light of these findings, many investigators believe that controlled clinical trials evaluating the impact of non-selective beta blockers (such as propranolol or carvedilol) on cancer are now warranted.

**Combination Regimens for Suppressing the Impact of cAMP on Cancer Spread**

Blocking one stimulus to cAMP generation in a cancer may have little therapeutic impact on a cancer if other stimuli are active as well. This suggests that possibility that concurrent administration of propranolol and cox-2 inhibitors might be more effective than administration of either one singly. To date, only one research group has reported on such a strategy. Glasner and colleagues showed that peri-surgical administration of propranolol and the cox-2 inhibitor etodolac to mice whose cancer-bearing paws were amputated significantly enhanced their subsequent survival, presumably because the stress of surgery had less impact on the survival/aggression of circulating cancer cells and on immune scavenging mechanisms; intriguingly, administration of either propranolol or etodolac alone was ineffective in this regard.\textsuperscript{135} However, long term concurrent administration of propranolol and a cox-2-inhibitor in rodent cancer models has not yet been reported. Current data are compatible with the interpretation that cox-2/PGE2 activity plays a prominent role in the onset of cancer, whereas adrenergic activity is a more compelling determinant of cancer progression.

A further strategy for lowering cAMP in cancers would be to employ agonists which inhibit adenylate cyclase via G\textsubscript{\alpha}\textsubscript{i}-linked receptors. GABA can achieve this effect in certain cancers – such as non-small-cell lung, pancreatic adenocarcinoma, hepatocarcinoma, cholangiocarcinoma, and breast cancer - via activation of GABA-B receptors.\textsuperscript{103;136-142} Al-Wadei and colleagues have reported that oral administration of GABA impedes the growth of non-small-cell lung cancer and pancreatic adenocarcinoma xenografts in mice, and complements the utility of celecoxib in this regard.\textsuperscript{35;136;138;139} These findings may be of some practical interest, as GABA is a nutraceutical that is well tolerated, owing to the fact that it does not penetrate the blood-brain barrier. Pharmaceutical agonists for GABA-B – which do penetrate the brain – are also available.

While adrenergic hormones, PGE2, and GABA seem likely to influence cAMP status in a broad range of cancers, other hormones doubtless impact cAMP production in certain specific cancers, and drug measures which modulate this signaling may thus have potential in the treatment of such cancers.

Finally, it is reasonable to predict that adding erlotinib (or other clinically available EGFR antagonists such as gefitinib, lapatinib, and the monoclonals cetuximab and panitumumab) to various regimens which
impede cAMP production in certain cancers could prove quite effective for suppressing the contribution of the cAMP/PKA/EGFR module to the survival and aggressiveness of these cancers.

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