cAMP-Mediated Regulation of CYP Enzymes and Its Application in Chemotherapy

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Abstract: Certain anti-cancer prodrugs are subject to cytochrome P450 (CYP)-mediated metabolism and become more active. Because CYP activity may be regulated by phosphorylation *via* adenylyl cyclase/protein kinase A, selective adenylyl cyclase subtype activators may be utilized in future chemotherapy to regulate CYP activity as a switch in a tumor tissue-specific manner.

Key Words: Cytochrome P450, protein kinase A, adenylyl cyclase, phosphorylation, forskolin, anti-cancer drugs.

CYP ENZYMES AND DRUG METABOLISM

A variety of metabolizing enzymes present in the liver and other organs can catalyze the reactions that can convert various xenobiotics, which are ingested into the body, to harmless or less harmless compounds [1, 2]. Such reactions are mostly performed by the mixed function oxygenase system, which is called cytochrome P450 (CYP), a key metabolic enzyme family [3]. In human, the CYP superfamily comprises 57 genes arranged in 18 families and 42 subfamilies as well as 46 pseudogenes [3]. These genes encode for enzymes involved in the metabolism of drugs, foreign chemicals, fatty acids, and cholesterol. Additionally, they play important roles in steroid synthesis and metabolism, bile acid as well as vitamin D synthesis and metabolism. Similarly, many ingredients in foods, as well as a number of toxicants, allergens, and carcinogens also serve as substrates for CYP. Mutations in many CYP genes cause inborn errors of metabolism, which may lead to increased risk of cancer or other diseases. It is also important to note that certain xenobiotics, which themselves are not carcinogenic, may be transformed by endogenous CYP into ultimate carcinogens, suggesting that CYP can generate more harmful compounds, which can be used for anti-cancer therapy under specific conditions.

REGULATION OF CYP BY cAMP/PKA

The enzymatic activity of CYP may change in response to various external stimuli, and the mechanisms for such changes are not specific to CYP, but rather similar to those for other enzymes [4-10]. The total amount of CYP may be increased, usually *via* induction at the level of gene transcription, or the enzyme catalytic activity *per se* may be changed *via* post-translational modifications, such as phosphorylation. While the former regulation may require several hours to days, the latter takes only seconds to minutes and is readily reversible, which is a major advantage of regulating the enzyme activity *via* phosphorylation.

Indeed, it is well known that CYPs are subject to regulation *via* phosphorylation [11]. The phosphorylation of essential components of CYP monooxygenase system, i.e., CYP and CYP reductase, was originally demonstrated using the catalytic subunit of protein kinase A (PKA). Later studies demonstrated that CYPs were phosphorylated not only *in vitro* (using purified kinases and CYPs), but in intact cells, as well as in whole animals. Importantly, CYP phosphorylation is highly isoenzyme-selective [12]. An example is CYP2 family [13], such as CYPB1/2B2 and CYP2E1 [14], which contains the consensus amino acid sequence for PKA-mediated serine/threonine phosphorylation. Because the phosphorylation of CYPs is a very fast process that occurs usually in seconds to minutes, and the phosphorylated proteins are inactivated immediately, it was suggested that the phosphorylation mechanism acts as a rapid switch to regulate CYP activity without changing the total amount of this enzyme, leading to dynamic changes in the control of the toxic metabolites of carcinogens as well as for the control of effectiveness of anti-cancer drugs [12], which are important issues in chemotherapy.

CYP-GENE TRANSFER AND ANTICANCER THERAPY

CYPs, most notably 1A, 1B, 2C, 3A, 2D subfamily members, are expressed not only in the liver, but in many tumor cells. For example, CYP1B1 is readily detectable in tumors, such as lung, breast, liver, gastrointestinal tract, prostate, and bladder tumor cells. Certain anti-cancer drugs, especially those in the form of prodrugs, are subject to CYP-mediated metabolism, and include alkylating agents (cyclophosphamide (CPA), ifosphamide (IFA), dacarbazine, procarbazine) and fluoropyrimidine. Some may become more active through CYP-mediated metabolism, and this mechanism may be used to activate certain anti-cancer drugs in a tumor tissue specific manner. For example, 2-(4-aminophenyl)benzothiazoles may be activated in CYP1A1 inducible tumors. CYP3A-mediated activation of AQ4N, an anticancer prodrug, into cytotoxic

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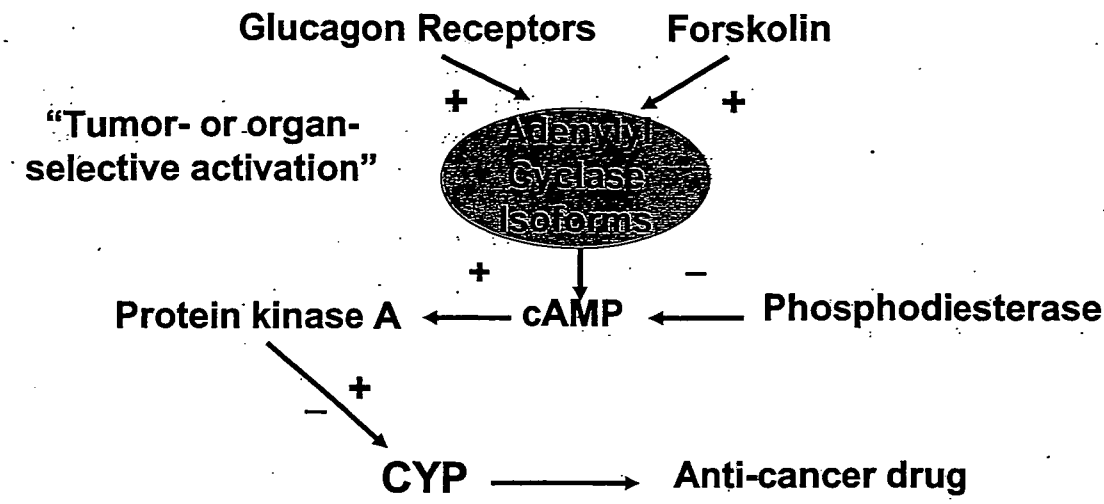

Fig. (1). A schematic concept for the proposed noble anti-cancer therapy.

metabolite may occur in hypoxic tumor cells [15]. CYP2B1 is induced by phenobarbital, a major mechanisms of CYP induction *in vivo*, and is responsible, together with CYP2C6 and CYP2C11, for conversion of the oxazaphosphorines CPA and IFA to their active metabolites [16, 17], while CYP2B1 is the most active catalyst. In human, in particular, analysis of 15 human CYP cDNAs expressed in human lymphoblasts and/or baculovirus-infected insect cells demonstrated that CYPs 2A6, 2B6, 3A4, 3A5, and three CYP2C enzymes (2C9, 2C18, 2C19) exhibited significant oxazaphosphorine 4-hydroxylase activity, with 2B6 and 3A4 displaying the highest activity toward CPA and IFA, respectively [18, 19]. These findings indicate that the expression of such CYPs are required for activating certain anti-cancer drugs.

If such CYP gene expression is not expressed in a tumor tissue, it can be introduced by gene transfer, i.e., anti-cancer gene therapy. CYP2B1 activates prodrug CPA into 4-hydroxycyclophosphamide, which ultimately degrades into acrolein and phosphoramide mustard, and serves as active (DNA-alkylating) metabolite. A recent study demonstrated that gene transfer encoding CYP2B1 indeed made tumor cells sensitive to this agent [20]. Further, such effects are not limited to tumor cells that were subject to gene-transfer. Diffusible cytotoxic metabolites can also inhibit the proliferation of surrounding tumor cells, in which the transgene was not introduced, a phenomenon called "the bystander effect". Indeed, it was demonstrated in cultured cells that CPA-sensitized, CYP-expressing C6 glioma cells transferred cytotoxicity to nonexpressing cells by releasing diffusible metabolites through the culture medium. This bystander effect occurred in the presence of CPA even when only a minor portion (~10%) of cells were gene-transferred [20]. Because the therapeutic efficacy and successful therapy with CPA is often limited by the fact that several tumor cells are not able to activate CPA or IFA, the above novel tumor-killing gene therapy with P450-based prodrug activation may be an effective method to improve the current anti-cancer therapy [21, 22] A very recent gene therapy trials using P450 provide strong support for the therapeutic potential of such gene therapy; treatment of inoperable pancreatic carcinoma patients with IFA in combination with encapsulated cells expressing CYP2B1 led to a 3-fold increase in 1-year survival [23].

REGULATION OF cAMP SIGNAL TO REGULATE CYP

It was recently demonstrated that the activation of PKA potentiated the pregnane X receptor (PXR)-mediated induction of CYP3A gene expression in cultured hepatocytes and increased the strength of PXR-coactivator protein-protein interaction [24]. This was demonstrated by the use of forskolin, a direct activator of adenylyl cyclase (AC), a membrane-bound enzyme that produces cAMP to activate PKA [25], suggesting that the induction of CYP can be achieved by regulating this cAMP producing enzyme, AC. In human, it is known that cAMP-dependent phosphorylation of CYPE1 leads to its changes in activity [14, 26].

If activation of AC is a strategy to activate of PKA, leading to the regulation of CYPs, is it possible to activate AC in a tumor tissue specific manner? Activation of AC may be made through the administration of glucagon, for example, when hepatic CYPs need to be regulated. Because glucagon receptors are expressed mostly, if not exclusively, in the liver, glucagon can activate PKA in a liver-specific manner. Other cAMP-regulating hormonal receptors, unfortunately, may not be expressed in a tissue-specific manner. Beta-adrenergic receptors, for example, that can potently stimulate the production of cAMP *via* activation of AC, are expressed in most organs, and thus the use of beta-adrenergic receptor agonist may activate PKA elsewhere. If a tumor tissue expresses a specific receptor subtype(s), agonist-medi-ated stimulation of such receptor can activate PKA in a tumor tissue specific manner. Unfortunately, the tumor tissue-specific expression of such a Gs-coupled receptor is not well known.

FORSKOLIN ANALOGUES AS TISSUE SPECIFIC ACTIVATOR OF PKA

If receptor activation may not be used, the elevation of intracellular cAMP and thus activation of PKA can be made by either the activation of AC, the cAMP producer, or the inhibition of phosphodiesterase (PDE), a key enzyme in the regulation of cAMP turnover. Because both AC and PDE

have multiple subtypes that differ in tissue distribution, a tumor cell may express a dominant subtype of either enzyme. Indeed, it is well known that AC and PDE subtype expression occurs in a much more tissue specific manner than receptor subtypes [27], and pharmacotherapy has already taken advantage of this property, and pharmacological compounds have been developed that can regulate AC and PDE in a subtype –specific manner. Subtype-specific PDE inhibitors, such as sildenafil citrate, a type 5 PDE inhibitor [28], and milrinone, a type 3 inhibitor [29], are now widely used in the treatment of erectile dysfunction and heart failure, respectively.

AC, which synthesizes cAMP, has at least 9 subtypes that differ in tissue distribution. Forskolin, a natural plant extract, was first identified as a general stimulator of AC, but a recent study has shown that 6-[3-(dimethylamino)propionyl] forskolin, a water-soluble forskolin derivative with high selectivity for type 5 AC, which is dominantly expressed in the heart, was developed and has been widely used in the treatment of acute heart failure [27, 30]. Furthermore, AC subtype specific inhibitors have been developed [31]. Adenine analogs or P-site inhibitors, which are classic, but not isoform-specific AC inhibitors, are now utilized to develop isoform-specific inhibitors [31, 32]. A novel non-nucleoside inhibitor, 2-amino-7-(2-furanyl)-7, 8-dihydro-5(6H)-quinazolinone (NKY80), was identified after virtual screening of more than 850,000 compounds by the use of crystallographic data of AC. NKY80 demonstrated a 210-fold selectivity for inhibiting type 5 AC relative to type 2 AC. Similarly, we found that some compounds, including 1R,4R-3-(6-aminopurin-9-yl)-cyclopentanecarboxylic acid hydroxyamide, potently inhibited type 5 AC, but not other subtypes [32], suggesting that various AC subtype specific inhibitors can be developed. Such efforts have been fortified by the development of computer-based screening for such subtype-selective compounds, which include the pharmacophore analysis using the crystal structure of enzyme protein. More recently, the first principle analysis, a method in theoretical physics, which analyses the molecular dynamics at the level of atoms and electrons, and has been widely used in simulation of developing new materials in semiconductor industry [33].

FUTURE DIRECTIONS

CYP, a key metabolic enzyme family for detoxification, is utilized in anti-cancer chemotherapy because it can generate, instead of detoxifying, more harmful compounds that can be used for anti-cancer therapy. Because the activity of some CYP can be regulated by AC/PKA, regulation of AC/PKA by the use of selective AC subtype activator may be utilized in future chemotherapy, which regulates CYP as a switch in a tumor tissue specific manner. Alternatively, a CYP member may be overexpressed by gene-transfer, and the activity of CYP may be regulated thereafter *via* the regulation of a AC subtype that is dominantly expressed in the tumor tissue. Nevertheless, these strategies would enable dynamic regulations in the control of the toxic metabolites and effectiveness of anti-cancer drugs in chemotherapy.

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