

# Perspective on Uddin

## Molecular Imaging of Inflammation and Carcinogenesis

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### Abstract

Development of imaging agents that can be used broadly for early detection of neoplasia at various tissue sites and at various stages of disease and that also can assess states of minimal residual disease would have tremendous utility in the diagnosis and management of cancer. In a series of articles culminating with a report in this issue of the journal (beginning on page 1536), Uddin and colleagues show their ability to *systemically* target the enzyme COX-2 with imaging probes that will serve as agents for early detection, risk assessment, prognosis, and intervention outcome measures. These probes will enable the detection and localization of regions of inflammation and a wide variety of premalignant lesions and cancers, with utility in monitoring the effects of cancer prevention and therapy. *Cancer Prev Res*; 4(10); 1523–6. ©2011 AACR.

There has been a dramatic increase in the number of imaging agents reported to have specificity for tumor-associated biomarkers. Many of these molecular targets are expressed on only a subset of tumors and are not broadly applicable. If all of the currently described promising agents could be translated to the clinic, the clinician would be faced with the problem of choosing which probes to use in each situation, unless the tumor had already been detected and molecularly typed. Developing and testing probes with specificity for more generalized markers of neoplasia that are associated with all stages of carcinogenesis are essential for effective imaging throughout the disease course. Such probes would have utility in early detection, assessing metastatic disease, and evaluating the outcome of prevention or therapy. Molecular probes for targets with more restricted expression can be used in conjunction with these probes for personalizing risk stratification, cancer prevention and therapy in high-risk and poor prognosis settings.

COXs are key enzymes in prostaglandin biosynthesis that are activated during inflammatory processes because of both the recruitment of inflammatory cells and increased expression in those cells. There are 3 genes encoding COX isozymes, *cox-1*, *cox-2*, and *cox-3* (inactive in humans), with COX-2 being the inducible form. The COX-2 enzyme is being evaluated as a target for cancer prevention and therapy as well as a biomarker with prognostic, diagnostic, and risk assessment potential. COX-2 converts arachidonic acid to

prostaglandin endoperoxide H<sub>2</sub> and is the target for the therapeutic/preventive effects of nonsteroidal anti-inflammatory drugs; COX-2-specific inhibitors are called coxibs. These agents have analgesic and antipyretic (fever reducing) effects and are anti-inflammatory at higher doses. The coxib family of drugs has been used in humans and for veterinary purposes. Celecoxib is used at present in a limited number of oncology applications. Other members of this family include etoricoxib, firocoxib, lumairacoxib, parecoxib, rofecoxib, and valdecoxib; several of these drugs are licensed for human use in the European Union but not in the United States. Although controversial, celecoxib is being evaluated for cancer chemoprevention and treatment on the basis of a strong body of data supporting the potential of COX-2 inhibition (1). The controversy relates to both the finding that long-term exposure to coxibs has been linked to the development of some cancers (2) and the significant risk of cardiovascular complications (1).

Regardless of the therapeutic uses of coxibs, the association of chronic inflammation with neoplasia combined with the correlation of elevated COX-2 levels with inflammation and premalignancy/cancer progression suggest that COX-2 is a useful imaging target and that coxibs may be modified for use as molecular probes for imaging early lesions. A number of examples, including Barrett's esophagus, intestinal-type gastric metaplasia, and breast premalignancy, show an association between COX-2 levels and chronic inflammation in premalignant conditions (3, 4). This association indicates that the assessment of COX-2 levels may have utility in assessing the predisposition to malignancy. Because COX-2 expression is elevated in many neoplastic cells (including breast, lung, skin, colon, ovarian, prostate, head and neck, and brain), its assessment also may have utility for early detection of cancer. Levels of COX-2 in neoplastic cells are prognostic for progression (5–7) and may be useful to predict drug efficacy (8, 9) and for assessing treatment response (10). Elevated COX-2 levels are seen in metastatic cells, and a causative role of COX-2 in the

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development of inflammation-related carcinoma and disease progression has been suggested.

Although the primary role of COX-2 in disease progression has yet to be fully elucidated, a number of mechanisms have been proposed. Expression of COX-2 has been shown to enhance survival and proliferation of malignant cells and to negatively influence antitumor immunity, enabling immune escape. High levels of COX-2 expression have been associated with tumor infiltration by FoxP3<sup>+</sup> T cells. This regulatory T-cell population is linked to immunosuppression within the tumor bed, which may prevent effective immunosurveillance (11). Chronic inflammation is a predisposing condition for most, if not all, cancers, and it seems logical that markers of inflammation (4, 12), particularly those that are also overexpressed in tumor cells, would be ideal targets for the early detection of cancer. Imaging agents are routinely used at subtherapeutic doses, which would circumvent the controversies in the field that relate to the safety of coxibs.

Both in biochemical assays and in cell culture, molecular targeting is greatly simplified relative to *in vivo* imaging with molecular probes. Analogous to drug development, the design of molecular probes for imaging needs to take into account the ability to cross biological barriers and optimal biodistribution. Once injected, a molecular probe must circulate for a reasonable period of time, cross biological barriers, and bind to selected targets with affinity and specificity, whereas the unbound molecular probe is removed by both diffusion and circulation. Long circulation times can be beneficial for therapeutic molecules, but high concentrations of circulating unbound molecular probe is undesirable in imaging because it leads to high background (i.e., "noise"). Therefore, modification of therapeutic compounds for use as imaging agents can be challenging, given the different pharmacokinetic properties that are useful for therapy versus for imaging. The coxibs, however, show very tight binding to COX-2 and have other properties that suggested they may be useful as molecular imaging agents.

COX-2 is an intracellular enzyme presenting unique challenges that are beyond those facing other molecular imaging targets. For example, targeting molecules that are present on the surfaces of the endothelial cells, such as agents based on the arginine-glycine-aspartic acid (RGD) peptide, is a strategy being advanced by a number of laboratories. Alternatively, other investigators are targeting markers that are present on the surface of tumor cells such as epidermal growth factor receptor (EGFR). Other molecular targets being developed as imaging targets are present in the extracellular space in the tumor bed such as matrix metalloproteinases. In contrast, COX-2 is a cytoplasmic enzyme located inside both immune cells and tumor cells. Therefore, to reach their target, COX-2 probes need to cross both the endothelial barrier and the plasma membrane of target cells. Moreover, to achieve molecular specificity for COX-2 over COX-1, the probes must selectively enter the active site of COX-2. This site is embedded in the enzyme beyond a constriction that is referred to as the "lobby"

(Fig. 1). Any molecular probe for COX-2 must also be small enough, therefore, to traverse this port of entry and then dock in the active site. The molecular composition of the probe cannot interact sterically or chemically with the amino acid residues that comprise the lobby region of the enzyme. Development of effective COX-2 probes for each imaging modality—optical, single-photon emission computed tomography, and positron emission tomography (PET)—required rational design of labeled forms of the COX-2 inhibitors. Modified indomethacin (13, 14) and celecoxib (15) were the 2 COX-2 inhibitors that showed specificity and utility in imaging studies; several molecular derivatives of these agents were critically evaluated by screening with biochemical, cellular, and *in vivo* assays (13, 14). This robust methodology is the strength of the work by Uddin and colleagues, as reported in this issue of the journal (15) and previously (13, 14).

This strength is most evident in Table 1 of the article by Uddin and colleagues (15), which shows the structures of each of the celecoxib derivatives that were evaluated as PET imaging agents and their selectivity for COX-2 over COX-1. A good example is in the comparison between compounds 7 and 10, which differ by 2 carbons in the linker domain used to fluorinate them. Despite the subtle differences in what should be an innocuous region of these molecules, they differ by more than 20-fold in their ability to inhibit COX-2 activity, relative to COX-1, in the biochemical assay.

An effective strategy used in this and the 2 prior studies by Uddin and colleagues was taking compounds from the design phase to *in vitro* or biochemical assays and then to assays in live cell cultures ("*in plastica*") and finally into several relevant *in vivo* models of human cancer. Each study was designed from the onset to consider the potential for *in vivo* measurements, with the aim to develop molecular probes that are effective in animal models and in humans. For these investigators, the *in vitro* and *in plastica* assays are viewed only as small steps in the development of effective molecular probes. Combining rational design and effective screening in relevant and diverse animal models increased the likelihood that the developed probes would be effective in humans. The term *in vivo* is defined differently in different fields. For chemists, studies using live cells in culture (*in plastica*) are often referred to as *in vivo* and enzyme assays as *in vitro*. In contrast, cancer researchers generally refer to cell culture studies as *in vitro* and studies in living animals and humans as *in vivo*. The term *in plastica*, or in cell culture, provides some clarity to these discussions. As chemists, Uddin and colleagues are to be commended on their use of *in vitro*, *in plastica*, and *in vivo* assays to test and validate their molecular probes.

The significance of this research is that the developed probes will have a breadth of uses that are analogous to PET imaging with fluorodeoxyglucose (FDG) in that the probes are specific for a molecular marker that is broadly expressed in neoplasia of many organ systems. The coxib probes, however, may go beyond FDG in their potential for early detection and as risk/prognostic indicators. For optical imaging, glucose has been labeled with fluorophores,



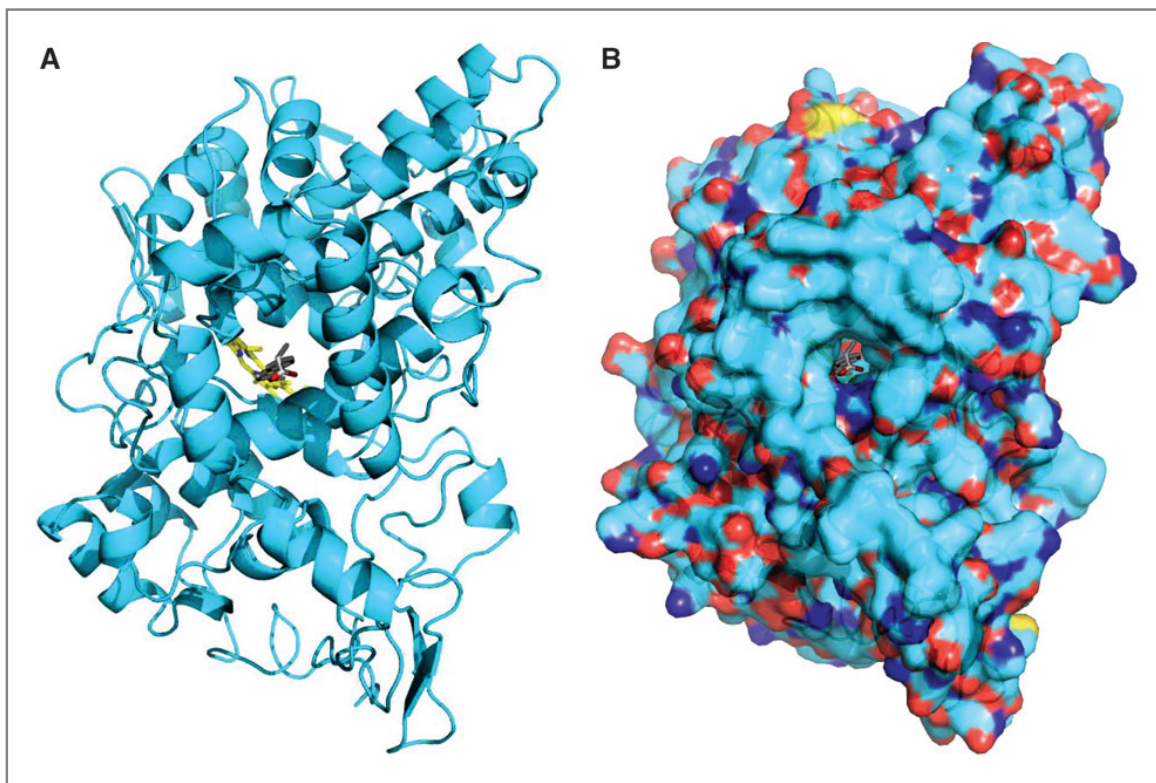


Figure 1. Binding of naproxen [(S)-6-methoxy methyl-2-naphthaleneacetic acid], a nonselective nonsteroidal anti-inflammatory drug, in the active site of COX-2. The crystal structure of COX-2, Protein Data Bank code 3NT1, is shown in cyan (greenish blue) and naproxen is shown as lines, gray for carbon and red for oxygen. COX-2 is a hemoprotein, and the heme molecule is shown in yellow for carbon, blue for nitrogen, and red for oxygen. A, a ribbon diagram of COX-2 looking through the lobby of the enzyme at its active site, where naproxen is bound (near the center of the image). B, same orientation of COX-2 but showing its molecular surface, which emphasizes the fact that the binding site is deep in the enzyme. Effective small-molecule probes for COX-2 activity need to enter the lobby and bind in the active site, as shown here for naproxen, and carry a label (e.g., a fluorophore or radioisotope) that can be detected outside the cell (cell culture) or from outside the body (*in vivo*). The colors of the molecular surface of COX-2 reflect carbon (cyan), nitrogen (blue), oxygen (red), and sulfur (yellow).

and Uddin and colleagues have similarly labeled coxibs for use in optical imaging and also for single-photon emission computed tomographic imaging (13–15). Such probes contribute greatly to our arsenal of imaging agents in that they provide broadly applicable markers of inflammation and cancer. This breadth will have utility in image-guided resections, where it is necessary to cast a broad net so as not to miss residual cancer cells. Detection of inflammation, metaplasia, and dysplasia as early predisposing conditions to malignancy are useful applications of these labeled coxibs. In addition, coxib probes may enable staging and assessing aggressiveness of a malignancy.

Because chronic inflammation can predispose tissue to develop malignancy, probes that highlight regions of inflammation may enable monitoring of high-risk premalignant lesions, direct the use of other molecular probes, and guide preventive interventions. Coxib probes highlight nonmalignant regions of inflammation to a lesser extent than they indicate COX-2 expression in tumor cells; therefore, these probes epitomize agents with broad utility. They can be used in early detection of cancer by revealing the

early lesions to indicate frank disease with greater signals and to enable evaluation of late-stage disease and response to therapy through changes in signals over time. Probes for targets with more restricted expression patterns will complement broadly active probes, such as those based on coxibs and FDG, by further refining the molecular characterization of cancers and guiding the use of (personalizing) molecular therapeutics. For example, more restricted probes are being developed for mutant EGFR in the lung (16, 17). Wild-type EGFR (such as COX-2) is overexpressed in many settings of neoplasia, and development of molecular imaging probes for wild-type EGFR is an active area of research (18).

The breast illustrates the potential broad application of the systemic use of the COX-2–targeted probes. Clinical studies show that COX-2 can be overexpressed at every stage of breast neoplasia, and there is no topical/optical means for imaging breast disease, a restriction that is also true for other internal organ sites. Furthermore, elevated levels of COX-2 in atypical hyperplasia (7) and ductal carcinoma *in situ* (5, 6) are associated with the risk of

progression to invasive cancer, and these levels in invasive cancer indicate a poor prognosis (19). COX-2 and inflammation in the breast are also associated with the increased expression and activity of aromatase (20), a key molecular target for prevention and therapy (21). Therefore, systemic COX-2-targeted probes could have utility in the detection of high-risk premalignancy and biologically aggressive early cancers, in addition to helping monitor preventive or therapeutic effects on breast neoplasia.

The voyage that Uddin and colleagues have taken with their molecular probes is as arduous as that of the molecular probe itself in a living subject with multiple possible barriers to success. Their work began with biochemical assays, transitioned into cells in culture, traveled through several animal models and through 3 imaging modalities and is now poised for clinical translation. This effort has resulted in molecular probes with *in vivo* utility that potentially will have a great impact on clinical cancer care by detecting neoplasia at different stages arising from different tissue origins and resulting from different molecular etiolo-

gies—truly a molecular probe for broad application in the clinic.

### Disclosure of Potential Conflicts of Interest

C. Contag is a founder of Xenogen Corporation (now part of Caliper LifeSciences) and a founder of ConcentRx Corporation. He has a financial interest in each of these companies. No potential conflicts of interest were disclosed by the other author.

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