ONCOLOGY AND INFLAMMATION

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WMIC Educational Course
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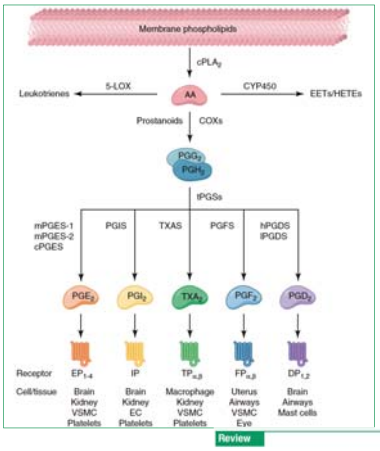
LEARNING OBJECTIVES

- Understanding the role of inflammation in the cancer phenotype
- Imaging inflammation and phenotypic changes induced by inflammatory pathways in cancer
- Image-guided targeting of inflammatory pathways in cancer

Inflammation (Latin, ɪnflammō, "I ignite, set alight") is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process.

Cancers have wound-like environments such as hypoxia and acidic extracellular pH – *'tumors are wounds that do not heal' (Dvorak, NEJM, 1986)*



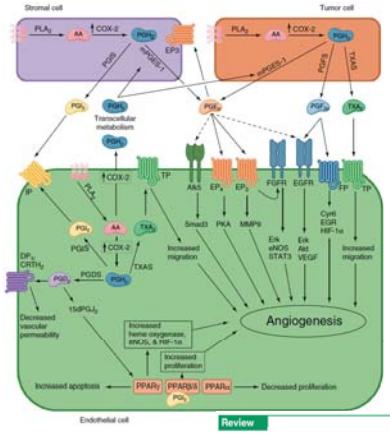
Prostanoid biosynthetic cascade. Arachidonic acid (AA) is released from membrane phospholipids by the hydrolyzing action of phospholipases.

AA is metabolized by lipoxygenases (LOXs) to form leukotrienes, by P450 isozymes to form epoxyeicosatrienoic acids, and by COX enzymes to form prostanoids.

The COX product PGH2 is further metabolized by specific synthases to yield prostaglandins and thromboxane, which bind prostanoid receptors to evoke a wide array of biological effects.

From: M. Dolores Salvado *et al.*,

Review
Trends in Molecular Medicine April 2012, Vol. 18, No. 4



Prostanoid metabolism reflects crosstalk between tumor, stromal and endothelial cells.

Prostanoids trigger angiogenic responses via binding to the EP, FP, and TP receptors and through the transactivation of EGFR (epidermal growth factor receptor), FGFR (fibroblast growth factor receptor) and Alk5 (TGFb receptor) present on nearby endothelial cells.

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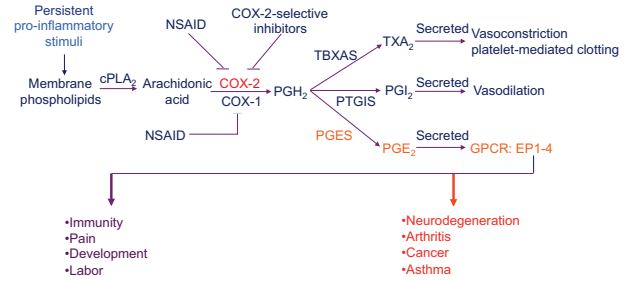
- The characteristic response of living vascularized tissue to injury is inflammation, which induces the formation of eicosanoids. Three well-known classes of phospholipases, phospholipase A2 (PLA2), phospholipase C (PLC) and PLD, participate in the formation of free arachidonate from membrane phospholipids in response to mechanical, chemical and physical stimuli.
- AA is converted to various eicosanoids by the action of lipoxygenases (LOX) and cyclooxygenases (COX). These eicosanoids impact on cell motility, invasion, vascular characteristics and metastatic dissemination.
- Most solid tumors, including breast cancers, exhibit inflammatory properties characterized by increased levels of prostaglandins and other proinflammatory molecules that are secreted by tumor cells, stromal cells, and specialized immune cells during inflammation.
- COX-1 (constitutive form) and COX-2 (inducible form) are cytoplasmic enzymes that convert PLA2-mobilized AA into the lipid signal transduction molecules prostaglandins and thromboxanes.
- One major product of the COX-2–catalyzed reaction is prostaglandin E2 (PGE2), an inflammatory mediator participating in several biological processes, including development, pain, immunity and angiogenesis, and cancer. COX-2 function has been the target of pharmaceutical intervention in a multitude of widespread degenerating conditions, including autoimmune diseases, gastric inflammation, and several different cancers, such as gastric, lung, breast, and colon cancer. Its expression is induced by proinflammatory cytokines, such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α, and its promoter contains a cyclic AMP response element, a nuclear factor-κB binding site, and two nuclear factors for IL-6 target sequences.

Studying the role of COX-2 in cancer



- Immunity
- Pain
- Development
- Labor

- Neurodegeneration
- Arthritis
- Cancer
- Asthma

NSAID: aspirin, naproxen, indomethacin
COX-2 selective inhibitors: celecoxib, rofecoxib, valdecoxib

COX-1 is not affected by the inflammatory process - gastroprotective
COX-2 is not detectable in normal tissue but is detectable after induction by inflammatory stimuli

Summary from Stasinopolous

COX-2 and cancer: a quick perspective

Search strings	Pubmed Hits
Inflammation cancer	20464
Hypoxia cancer	8335
Angiogenesis cancer	23375

Search strings	Pubmed Hits
COX-2 cancer	5120
HIF cancer	3191
VEGF cancer	13549

Source: pubmed

COX-2 selective inhibitor celecoxib in clinical trials:
309 past, present or planned
174 in cancer
23 recruiting

Source :www.clinicaltrials.gov

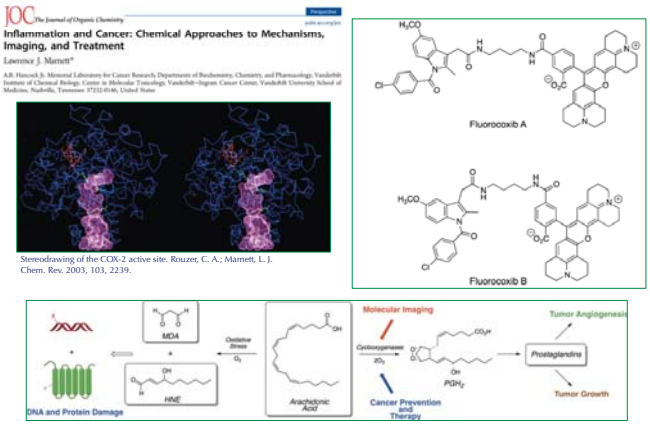
Celecoxib is given as chemoprevention in patients with Familial Adenomatous Polyposis

Selective COX-2 inhibitors are thought to increase the risk of adverse cardiovascular reactions

COX-2 is expressed in *ca.* 40% of primary breast tumors

Clinical inflammation (edema) is not necessarily required for COX-2 expression in the microenvironment

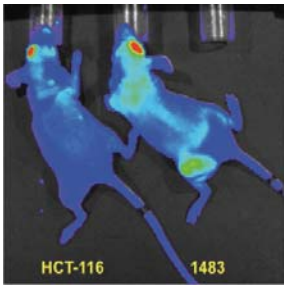
From: Summary from Stasinopolous



Stereodrawing of the COX-2 active site. Rouzer, C. A.; Marnett, L. J. Chem. Rev. 2003, 103, 2239.

J. Org. Chem. 2012, 77, 5224−5238

Accumulation of fluorocoxibs in the inflamed paw. Carageenan was injected into the paw at time zero. After 24 h, the fluorocoxib was administered by intraperitoneal injection.



Xenograft data on uptake of fluorocoxib A. Fluorocoxib A was administered by retro-orbital injection and the animals monitored for fluorescence 3.5 h later using a Xenogen camera. The head-and-neck cancer, 1483, expresses COX-2 whereas the colon cancer, HCT116, does not express COX-2.
From: Uddin, M. J. *et al.*, Cancer Res. 2010, 70, 3618.

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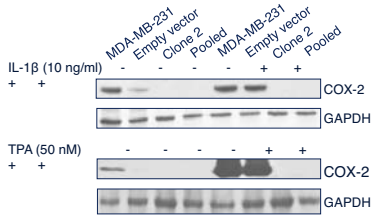
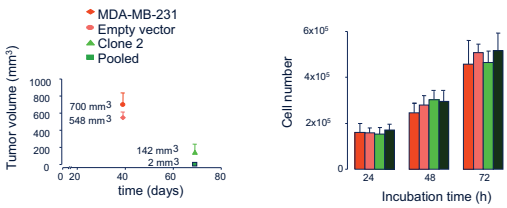
Silencing of COX-2 in MDA-MB-231 cells



Clone 2: Clone stably transfected with a plasmid coding for a COX-2 shRNA
Pooled: Pool of four clones stably transfected with the COX-2 shRNA plasmid

I. Stasinopoulos *et. al. Mol Cancer Res.* 5, 435 (2007)

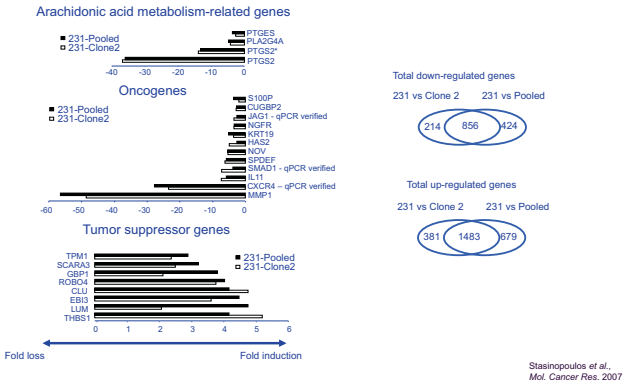
Silencing of COX-2 delays tumor onset in SCID mice



	number of mice	measurable tumors (30 days)	measurable tumors (60 days)	measurable tumors (90 days)
MDA-MB-231	10	10*/10	n/a	n/a
Empty vector	9	9*/9	n/a	n/a
Clone 2	11	0/11	1*/11	1/10
Pooled	10	0/10	0/10	4/10

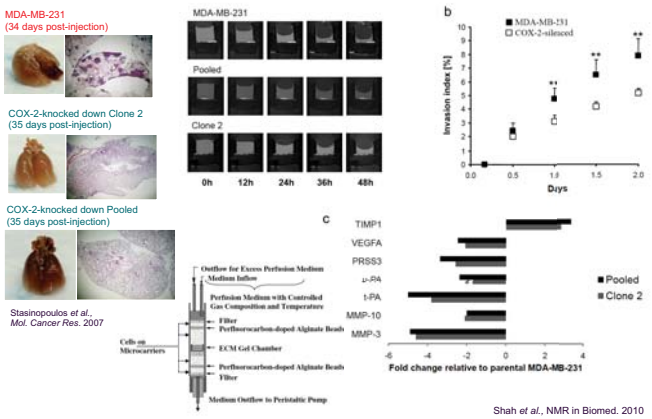
Stasinopoulos *et al.*, *Mol. Cancer Res.* 2007

COX-2 silencing alters the transcriptome of MDA-MB-231 cells



Stasinopoulos *et al.*, *Mol. Cancer Res.* 2007

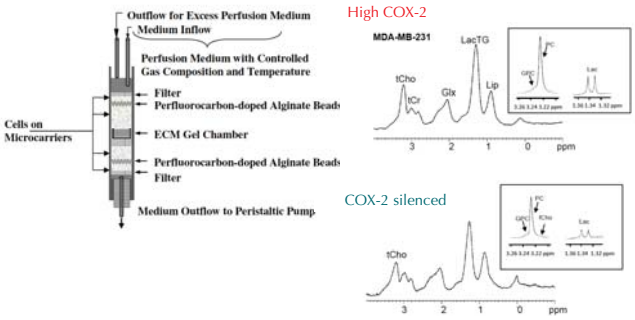
COX-2 SILENCING INHIBITS INVASION AND METASTASIS



Stasinopoulos *et al.*, *Mol. Cancer Res.* 2007

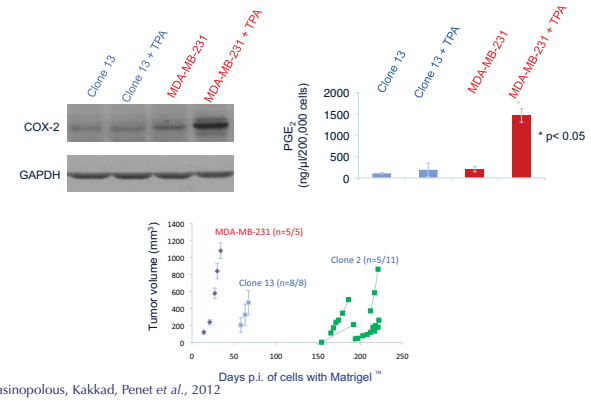
Shah *et al.*, NMR in Biomed. 2010

Proton Spectroscopy

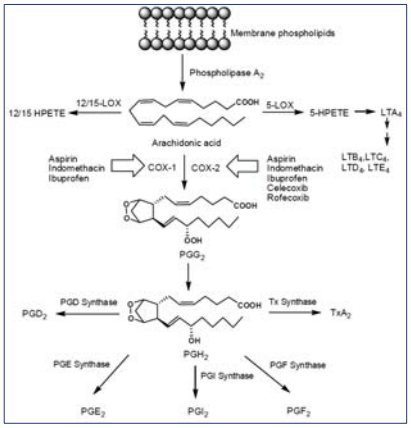


Shah *et al.*, NMR in Biomed. 2010

COX-2 expression and PGE_2 production are reduced, but not silenced in Clone 13 cells



From: Stasinopolous, Kakkad, Penet *et al.*, 2012



Targeting COX

J Pharm Pharmaceut Sci 11 (2): 81s-110s, 2008
Evolution of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs): Cyclooxygenase (COX) Inhibition and Beyond
P. N. Praveen Rao1 and Edward E. Knaus2.

Targeting COX

- Greek physician Hippocrates (c. 460 BC – c. 370) prescribed an extract from willow bark and leaves. In the 17th century, the active ingredient of willow bark salicin was identified in Europe. The Kolbe company in Germany started mass producing salicylic acid in 1860.
- Acetylsalicyclic acid 1 (Aspirin) was introduced by Bayer in 1899.
- John Vane (in the seventies) discovered the mechanism of action of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs).

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Targeting COX – non specific effects

Class	Properties	Examples
Group 1	NSAIDs that inhibit both COX-1 and COX-2 completely with little selectivity	Aspirin, ibuprofen, diclofenac, indomethacin, naproxen, piroxicam
Group 2	NSAIDs that inhibit COX-2 with a 5-50 fold selectivity	Celecoxib, etodolac, meloxicam, nimesulide
Group 3	NSAIDs that inhibit COX-2 with a > 50 fold selectivity	Rofecoxib, NS-398
Group 4	NSAIDs that are weak inhibitors of both isoforms	5-Aminosalicylic acid, sodium salicylate, nabumetone, sulfasalazine

GI side effects associated with traditional NSAIDs are due to the inhibition of gastroprotective PGs synthesized via the COX-1pathway

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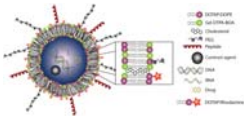
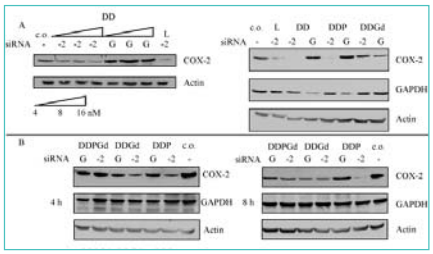
Imaging of cationic multifunctional liposome-mediated delivery of COX-2 siRNA

M Mikhaylova[1], I Stasinopoulos[1], Y Kato[1], D Artemov[1,2] and ZM Bhujwalla[1,2]

Cancer Gene Therapy (2009) 16, 217–226

www.nature.com/cgt





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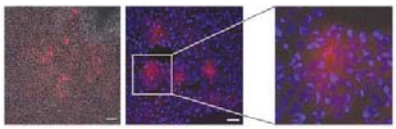
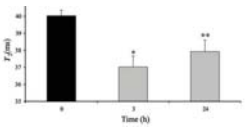
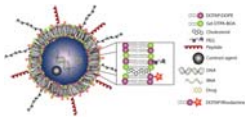
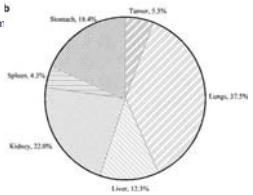




DY647-labeled cyclooxygenase-2 (COX-2) DDP lipoplexes localization in fresh tumor sections) at 8 h after iv injection. Left panel: phase-contrast images overlayed with fluorescence images. Left column: 10 objective, bar 50 mm; middle column: 40 objective, bar, 20 mm and right column: pixel-level magnification.





Acknowledgements

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