COX-2-dependent regulation of cytokine balance and apoptosis resistance in non-small cell lung cancer

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Steven Dubinett, M.D.
UCLA Lung Cancer Research Program
Prostaglandin (PG) Pathway

Arachidonic Acid

- Cyclooxygenase-1
- Cyclooxygenase-2

PGG$_2$

Peroxidase

PGH$_2$

PG Synthases

TXA$_2$, PGD$_2$, PGE$_2$, PGI$_2$, PGF$_{2\alpha}$

NSAIDs (1-5 $\mu$M)

COXIBs
COX-1 Versus COX-2

Arachidonic Acid

**COX-1**
(Constitutive)
- Platelets
- Stomach
- Intestine
- Kidney

**COX-2**
(Inducible)

NSAIDs

Inflammatory Sites:
- Macrophages
- Endothelial cells

Selective COX-2 inhibitors
COX-2 in Lung Cancer

- Overexpressed in NSCLC and pre-neoplasia
  
  Huang M, Ca Res 1998; Wardlaw S, Carcinogenesis 2000; 
  Hosomi Y, Lung Ca 2000 )

- A marker of poor prognosis in stage I NSCLC
  
  (Khuri F, Clin Ca Res 2001; Achiwa H, Clin Ca Res 1999)

- Induced by tobacco carcinogens
  
  (Yan Z, JBC 2000; Rioux N, Inflam Res 1999)

- Preclinical studies of COX-2 inhibitors suggest antitumor and chemopreventive efficacy
  

- Epidemiologic data suggest subjects who routinely use NSAIDs have decreased lung cancer risk
  
  (ie Akhmedkhanov Brit J Cancer 2002)
Rationale for COX-2 Inhibition in Lung Cancer

• **Induces apoptosis and enhances cytotoxicity of anticancer agents** (Hida T, Clin Cancer Res 2000)

• **Induces antiangiogenic effects in lung cancer models** (Masferrer J, Ca Res 2000)


• **Decreases tumor invasiveness** (Dohadwala M, J. Biol Chem 2001 & 2002)
Survival of Patients with NSCLC

Estimated probability of survival

Survival in months

Low COX-2 expression

High COX-2 expression

P=0.0032

Brabender et al. *Ann of Surg* 2002;235:440
How does COX-2 regulate the malignant phenotype in non-small cell lung cancer?
COX-2-dependent Modulation of the Malignant Phenotype

- Angiogenesis
- Invasion
- Resistance to apoptosis
- Immunosuppression

TUMOR COX-2
Lung Cancer-mediated Inhibition of Antitumor Immunity

- Lack of costimulatory molecules
- Production of immune inhibitory mediators

\[ \text{PGE}_2, \text{TGF-} \beta, \text{IL-}10, \text{VEGF} \]
COX-2-mediated inhibition of antitumor immunity in NSCLC

Huang et al, Cancer Research 1998; 58:1208
Stolina et al, Journal of Immunology 2000; 164: 361
Specific Inhibition of Tumor COX-2 Limits Tumor Growth \textit{in vivo}

Stolina et al J Immunology 2000
No treatment

Indomethacin

Celecoxib

Resveratrol

Anti-PGE2

Indomethacin

COX-2 antisense

Tumor volume (mm³)

Days

0 6 12 18
COX-2 Inhibition in vivo Reduces PGE\textsubscript{2} and IL-10 Production by *Tumor Tissues*

(ng/ml/g of tumor tissue)

<table>
<thead>
<tr>
<th>3LL tumors from:</th>
<th>IL-10</th>
<th>PGE\textsubscript{2}</th>
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<tbody>
<tr>
<td>Untreated mice</td>
<td>734 ± 74</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>COX-2 Antisense</td>
<td>184 ± 27*</td>
<td>11 ± 2*</td>
</tr>
<tr>
<td>Celecoxib-treated</td>
<td>398 ± 38*</td>
<td>17 ± 1*</td>
</tr>
<tr>
<td>Indomethacin-treated</td>
<td>268 ± 60*</td>
<td>12 ± 1*</td>
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</table>

* p<0.05 compared to untreated tumor bearing mice.*
IL-10 overproducing T cells reverse the COX-2 inhibitor anti-tumor response
COX-2-mediated inhibition of antitumor immunity in NSCLC

TUMOR COX-2

+ +

PGE2

+ +

IL-10

lymphocytes

APC

 IL-12
COX-2-mediated inhibition of immune-dependent angiogenesis

1. PGE2
2. COX-2
3. TUMOR

Decrease in anti-angiogenic chemokines
- Interferon –inducible protein 10 (IP-10)
- Monokine induced by IFN\(\gamma\) (MIG)

Interactions:
- PGE2 → IL-12 → IFN\(\gamma\)
COX-2-mediated inhibition of immune-dependent angiogenesis

- Decrease in anti-angiogenic chemokines
  - Interferon–inducible protein 10 (IP-10)
  - Monokine induced by IFNγ (MIG)
Biology of COX-2 Inhibition in Cancer

- How can we further our understanding of the impact of COX-2 expression in human lung cancer?
- What biomarkers should we monitor in trials that utilize COX-2 inhibitors?

Note: trials reported by David Johnson (ASCO 2003), Nasser Altorki (JCO 2003), and Jenny Mao (Clin Cancer Res 2003) demonstrating decreased PGE$_2$ following celecoxib in NSCLC patients
What COX-2-dependent Genes/Proteins Mediate the Malignant Phenotype in NSCLC?
What COX-2-dependent Genes/Proteins Mediate the Malignant Phenotype in NSCLC?

**Tumor invasion**: CD44 and MMP2  
Dohadwala et al, JBC 2001, 276: 20809 &  
JBC 2002, 277:50828

**Immune regulation**: IL-10 and IL-12  
Huang et al, Cancer Res 1998, 58:1208

**Angiogenesis**: CXCL5, CXCL8, VEGF  
Pold et al  Cancer Research 2004, 64(5):1853-60

**Apoptosis**: survivin, IGF-BP-3, IL-6  
Pold et al Cancer Res 2004, 64: 6549
NSCLC COX-2-dependent Modulation of the Malignant Phenotype

- Angiogenesis
- Invasion
- Resistance to apoptosis
- Immunosuppression
COX-2 and STAT3 in NSCLC

IL-6

COX-2

CD130

IL-6Rα

STAT3

P

P

NSCLC

Anti-Apoptotic

(Bcl-2, Mcl-1, survivin)

Proliferation

Angiogenesis

(VEGF)

Tumor progression
COX-2-dependent Apoptosis Resistance in NSCLC Cells

A549

Control
COX-2 Inhibited
COX-2 Over-expressed

Cell death, %

Not irradiated
Irradiated

* p<0.0001, ** p<0.005 vs control

Krysan et al FASEB Journal Jan 2004
Survivin

- A member of Inhibitor of Apoptosis Proteins (IAP) family
- Binds caspases
- Frequently over-expressed in human malignancies
- Over-expression is associated with poor prognosis in NSCLC
COX-2-dependent survivin expression in COX-2 gene-modified NSCLC cells

Krysan et al FASEB Journal Jan 2004
Co-expression of COX-2 and Survivin in Lung Adenocarcinoma (A, B, C) and Squamous Cell Carcinoma (D, E, F)

Krysan et al FASEB Journal Jan 2004
Co-expression of COX-2 and Survivin in Lung Adenocarcinoma and Squamous Cell Carcinoma
Inhibition of survivin expression with siRNA significantly reduces apoptosis resistance of H157S NSCLC cells

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<thead>
<tr>
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<th>H157P</th>
<th>H157S</th>
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<tr>
<td>Control siRNA</td>
<td>Survivin siRNA</td>
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<td>Survivin siRNA</td>
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Upper sections — no apoptosis induction
Lower sections — 6 µg/ml Camptothecin~18 hours
Survivin Conclusions

1. Overexpression of COX-2 as well as treatment with PGE2 significantly increases the apoptosis resistance of NSCLC cells.

2. Survivin expression is COX-2-dependent in NSCLC cells and its level is lowered by COX-2 specific inhibitors.

3. Ubiquitination of survivin is blocked by high levels of COX-2 and PGE2.

4. Survivin and COX-2 are frequently co-expressed in human NSCLC.

Krysan et al FASEB Journal Jan 2004
NSCLC COX-2-dependent Modulation of the Malignant Phenotype

- Angiogenesis
- Invasion
- Resistance to apoptosis
- Immunosuppression
COX-2 Regulates Pro-angiogenic Chemokine Expression in NSCLC

**A549**

**H157**

NSCLC COX-2-dependent Modulation of the Malignant Phenotype

- Angiogenesis
- Invasion
- Resistance to apoptosis
- Immunosuppression
The receptor for hyaluronate, plays an important role in regulating tumor growth and metastases because it mediates adhesion to ECM.

Dohadwala et al, J. Biol Chem. 2001, 276: 20809
J. Biol Chem. 2002, 277: 50828
COX-2-dependent Invasion of NSCLC is Mediated by CD44 Expression

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<td>IL-1β</td>
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PGE2 mediated autocrine/paracrine signaling

NSCLC

COX-2

PGE2

AA

PGH2

Paracrine

Autocrine

Invasion, angiogenesis, apoptosis resistance

Neighboring Cells
E-cadherin stains red; COX-2 brown
COX-2
An Injury Response in Lung Epithelium

Cytokines
Carcinogens
Hypoxia
Chemotherapy
Mutations (K-ras)

Epithelial cells
COX-2
An Injury Response in Lung Epithelium

Cytokines
Carcinogens
Hypoxia
Chemotherapy
Mutations

Epithelial cells

PGE 2
# UCLA Lung Cancer Research Laboratory

<table>
<thead>
<tr>
<th>Faculty</th>
<th>Trainees and Staff</th>
<th>Collaborators</th>
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<tbody>
<tr>
<td>Sherven Sharma</td>
<td>Mariam Dohadwala</td>
<td>Robert Strieter</td>
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<tr>
<td>Raj Batra</td>
<td>Felicita Baratelli</td>
<td>Mitchell Kronenberg</td>
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<td>NIH P50 CA90388</td>
<td>Harnisha Dalwadi</td>
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