



Apricoxib, a novel inhibitor of COX-2, enhances the efficacy of standard therapy in human pancreatic cancer cell lines in vitro and in vivo

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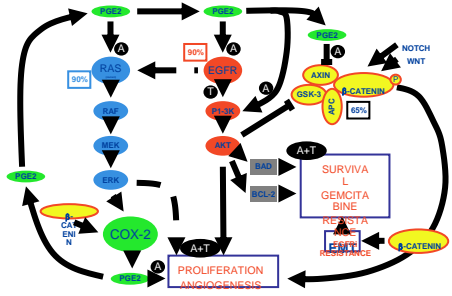


Abstract

Gemcitabine-erlotinib is standard of care (SOC) for pancreatic cancer, but this regimen can still be improved upon with regards to prolonging patient survival. Cyclo-oxygenase-2 (COX-2) is overexpressed in pancreatic cancer & implicated in pancreatic tumor progression. Inhibition of COX-2 decreases tumor growth, augments the activity of both gemcitabine and EGFR inhibition and may play a role in preventing or reversing EMT. This study characterized the effects of COX-2 inhibition on pancreatic cancer cell lines and determined if the addition of apricoxib enhances sensitivity to chemotherapy. Baseline EGFR and COX-2 expression were determined in 7 human pancreatic cancer cell lines & functional responses measured by changes in phospho-EGFR (p-EGFR) and prostaglandin E2 (PGE2) production by ELISA. Cytotoxicity was determined for gemcitabine, erlotinib & apricoxib independently and in combination by MTS assay. The effect of SOC therapy alone or in combination with 10 or 30mg/kg apricoxib PO on AsPc-1 & Colo357 tumors in vivo was determined in SCID mice bearing established orthotopic xenografts. Tissue was analyzed by IHC & VEGF levels were determined by ELISA. All cell lines expressed EGFR and COX-2, but expression alone was not predictive of p-EGFR level, PGE2 production or response to drug therapy. In vitro, AsPc-1 cells had negligible COX-2 activity, whereas Colo357 cells displayed high levels of COX-2 and PGE2 production. Cell growth and COX-2 activity decreased in all cell lines in the presence of apricoxib and addition of apricoxib improved response to chemotherapy. In vivo, addition of apricoxib to SOC significantly reduced primary tumor growth & almost eradicated metastases in mice bearing Colo357 but not AsPc-1 xenografts. Plasma VEGF levels were unaltered by apricoxib treatment in AsPc-1-bearing animals but were suppressed to undetectable levels in Colo357-bearing animals. Markers of EMT were significantly decreased in animals treated with apricoxib. Apricoxib enhances the efficacy of gemcitabine and erlotinib in vitro and in vivo & warrants clinical evaluation in patients with pancreatic cancer. A Phase 2 study is ongoing.

Background

- Pancreatic cancer:**
 - 5 yr survival <5%, highly resistant to most therapies
 - Current standard of care, Gemcitabine and Erlotinib only improves overall survival from 5.9 months to 6.2 months
 - COX-2:**
 - Expressed in up to 75% of pancreatic cancers
 - Use of ASAs/NSAIDs is associated with decreased incidence of pancreatic cancer
 - Inhibition of COX-2 may potentiate the benefits of chemotherapy
 - Currently available COX-2 inhibitors are prohibitively toxic for use in cancer therapy
- Molecular basis of synergistic effects of erlotinib, apricoxib and gemcitabine in pancreatic cancer**

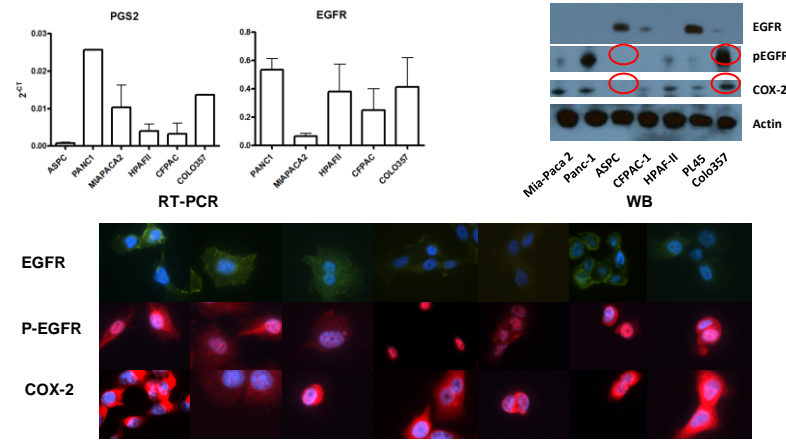


Methods

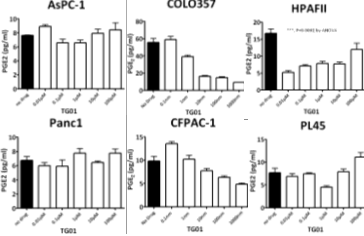
- PDAC cell lines MIA PaCa-2, PANC-1, CFPAC-1, AsPc-1, Su.86.86, HPAF-II, PL45, and Colo357 were used to evaluate in vitro effects of Apricoxib, Erlotinib, and Gemcitabine
- Baseline expression of Cox-2, EGFR, and p-EGFR were determined by ICC, Western blot, PCR, and ELISA.
- Functional change in phosphorylation of EGFR and PGE2 production in the setting of Erlotinib and apricoxib, respectively, were determined by ELISA.
- MTS assay was used to evaluate changes in cell proliferation with each drug and combination therapy.
- A COX-2 negative and COX-2 expressing cell line were selected for orthotopic injection in SCID mice to compare standard therapy to combination with apricoxib
- VEGF levels were determined by ELISA. MVD, vessel maturity, proliferation, apoptosis, and EMT markers were evaluated by IHC

Results

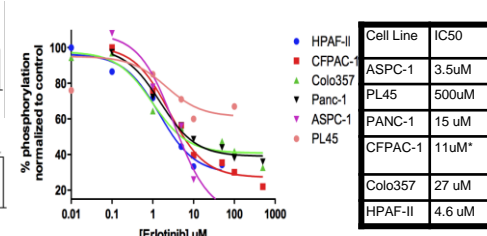
Expression of COX-2 and EGFR in vitro



Effect of apricoxib on PGE2 levels



Effect of Erlotinib on p-EGFR

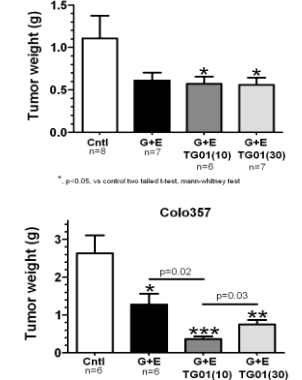


Changes in sensitivity to Gemcitabine with Combination therapy

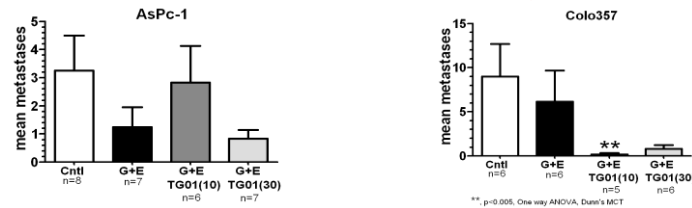
	E	A	Gem	G+1uM E	Gem + A 0.15uM	Gem + A 1uM	Gem+1uM E +1uM A
AsPc-1	70	375uM	2000nM	1630nM	2000nM	830nM	537nM
Colo357	400uM	400uM	419nM	92nM	116nM	86nM	52nM
HPAF-II	80uM	400uM	2000nM	172uM	2000nM	400nM	13nM
Panc-1	2.3uM	23uM	2000nM	*	2000nM	2000nM	2000nM
CFPAC-1	4.3uM	19uM	2.7nM	*	2.1nM	2nM	0.72nM
PL45	26uM	7uM	12.8nM	*	24nM	9.1nM	7nM
Mia PaCa	400uM	400uM	24nM	*	18nM	26nM	18nM
Su.86.86	400	34uM	17nM	*	*	*	10nM

*maximum dose Gem 2000nM, Erlotinib 400uM, Tg01 400uM

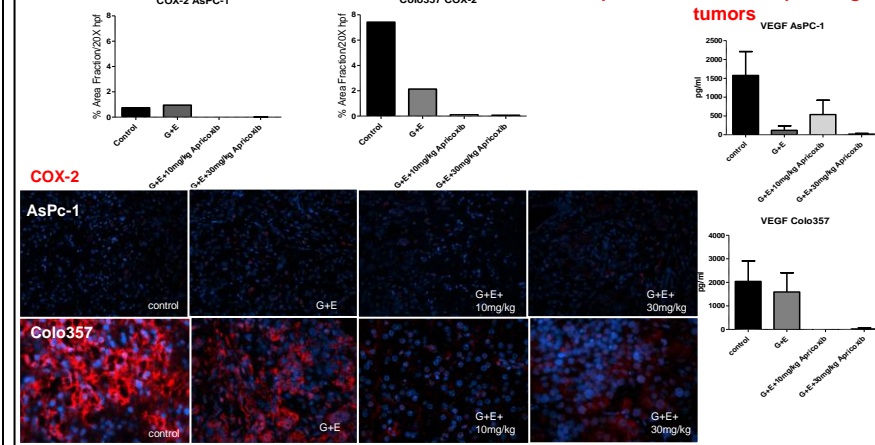
Apricoxib improves efficacy of standard therapy in vivo



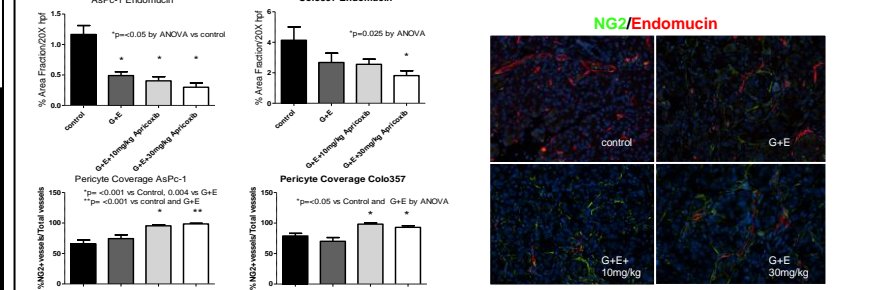
Apricoxib reduces metastatic incidence in COX-2 expressing tumors



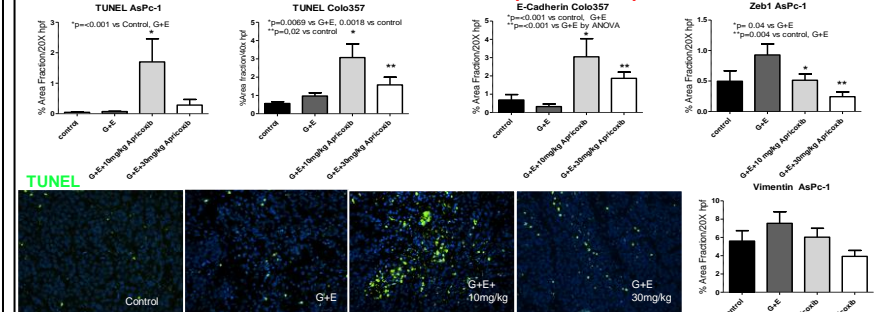
COX-2 expression is low in AsPc-1 tumors, decreased VEGF production is decreased with Apricoxib in COX-2 expressing tumors



Microvessel density decreases, vessel maturity increases in Apricoxib treated tumors



Apricoxib treatment increases tumor cell apoptosis



Conclusion

- All cell lines express EGFR and Cox-2, Baseline expression of EGFR and Cox-2 is not directly predictive of degree of activity of downstream products nor response to drug therapy
- Addition of apricoxib to standard therapy reduces tumor growth, this effect is greater in COX-2 over-expressing tumors
- In COX-2 over-expressing tumor cell lines, combination therapy dramatically reduced metastatic incidence
- Inhibition of COX-2 reduces host VEGF production and microvessel density while increasing pericyte coverage of vessels
- Inhibition of COX-2 induces apoptosis and may prevent or reverse EMT
- COX-2 activity and pancreatic cancer cell growth are inhibited by apricoxib providing an additive anti-tumor effect when combined with Gemcitabine and Erlotinib. Further clinical evaluation is warranted, a phase II study is ongoing.