Differential Protein Expression in Small Intestinal Carcinoids and Liver Metastases

Michelle Kang Kim, Daguan Wang, Miao Cui, Thomas Curran, Stephen Ward, Richard Warner, Sasan Roayaie, Michael Shafir, Myron Schwartz, Max Sung, David Zhang, and Steven Itzkowitz

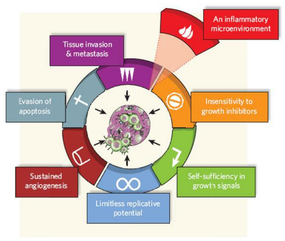
Divisions of Gastroenterology, Surgical Oncology, Oncology and Gastrointestinal Pathology. Mount Sinai School of Medicine, New York, NY 10029

BACKGROUND/AIMS

- Small intestinal carcinoids often have heterogeneous behavior that may present clinical challenges.
- Often detected incidentally on cross-sectional imaging as well as in symptomatic patients, these lesions may present with different biologic behavior.
- Because of our limited understanding of the natural history of these lesions, it would be helpful to understand important pathways in tumor growth and metastasis development.
- Investigators at our institution have recently developed a proteomic based approach, termed Protein Pathway Array (PPA), which allows global screening of changes in protein expression and post-translational phosphorylation.

Broad coverage of cellular pathways:

- Angiogenesis
- Apoptosis
- Inflammatory-response cytokii
- Hormone receptor signaling
- Cell proliferation pathways
- Cancer drug resistance
- Chemokines and receptors
- Cell damage and repair
- Drug metabolism
- Extracellular matrix and adhes
- Hypoxia signaling pathway
- Stem cells
- Stress response
- Tumor metastasis



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- The focus of the PPA is to determine the signaling network that controls cancer development. Thus, when particular proteins are identified to be up-or down-regulated, these findings can be immediately placed into context with known pathways that carry the potential for therapeutic intervention.
- The Aim of our study was to identify key pathways that may be important in the development of SIC metastases.

METHODS

PATIENTS: Patients undergoing surgical resection of SICs with metastases to liver

TISSUES: Tissue from liver metastasis, paired primary SIC compared to matched tissue controls from same patient of normal liver and normal small intestine

METHODS (cont)

•Extracted proteins were separated on an SDS gel and blotted on a multichannel manifold with 136 antibodies.

•Positive bands were identified and band densities were determined using BioRad Image system.

•Significant Analysis of Microarray (SAM) (http://www-stat.stanford.edu/~tibs/SAM/) was used to select the proteins differentially expressed between different groups.

•Unsupervised hierarchical clustering analysis was performed using BRB Array Tools software v.3.3.0 (htt://linus.nci.nih.gov/BRB-ArrayTools.html).

RESULTS

- Of the 136 proteins analyzed, 52 proteins were expressed in these samples.
- 9 proteins were up-regulated in primary SICs compared with matched normal small bowel mucosa (Table 1).
- Cyclin E was downregulated in primary SIC tissue compared to normal small bowel mucosa.
- Compared to normal liver tissue, SIC liver metastases demonstrated up-regulation of P-ERK and p27 but down-regulation of CDK2 and CDC25B.
- Curiously, when comparing primary SIC with their paired liver metastases, cyclin E demonstrated a significant upregulation in the liver metastasis.

Protein	Ratio: Tumor/Normal (fold change)	SAM (q%)
(a)		
p-PDK1	3.41	4.44
PTEN	21.47	4.44
cdk4	8.53	0.00
MetRS	8.53	0.00
P27	11.95	0.00
XIAP	9.75	0.00
P38	9.66	0.00
Stat 3	10.38	0.00
Alpha-tubulin	11.90	0.00
Cyclin E	0.15	6.67
(b)		
p-ERK	5.27	0.00
P27	9.61	0.00
Cdk2	0.56	0.00
Cdc25B	0.02	0.00
(c)		
Cyclin E	3.48	0.00

Table 1. Up and down regulation of proteins in small bowel primary carcinoid and liver metastases. (a) Comparison of SIC primary carcinoid vs normal small bowel. (b) Comparison of SIC liver metastasis vs normal liver. (c) Comparison of SIC liver metastasis and primary carcinoid.

RESULTS (cont.)

Appendix: Selected proteins/phosphoproteins used in Pathway Array

Angionesis: FGF, EPO, VEGF, VEGFR, NRP-1, Ang1, PDGF, PDGFR, TGF-beta, TGF-beta Receptor, Endoglin, VE-cadherin, CD31, NOS, COX-2, Id1/Id3, AC133, Angiopoietin, Laminin, Stabilin, Neuropilin, KDR, FLT1, IL8, Prokineticin, TNF alpha, IFN, IL6, COX1, MMP2, MMP9
Apoptosis/Autophagy: Bax, FAS, BAD, BCL2, BIK, BID, BAK, cleaved Caspase 3, cleaved Casepase 7, cleaved Caspase 8, cleaved Caspase 9, TRAF, FADD, TRADD, p53, CD27, BRAF, PARP, XIAP, NFKB, IKB, TAK1, RIP1, Bcl-xL, FLIP, Smac, Cytochrome C, Apaf-1, LC-3I, LC-3II, Raptor, VPS15, Beclin1, TNF
Cell signaling: ERK1/2, p-ERK1/2 (Thr202/Tyr204), Akt, p-AKT (Ser473), HGF, HGFR, pHGFR (Y1234/Y1235), IGF, IGFR, TGF, TGFR, Notch 4, Notch 1, p38, p-p38 (Thr180/Tyr182), JNK, p-JNK (Thr183/Tyr185), FGFR, p-FGFR (Tyr653/654), VEGFR, p-VEGFR (Tyr951), PKC, p-PKCalpha (Ser657), p-PKCalpha/beta (Thr638/641), PTEN, p-PTEN (Ser380), PI3K, Ras, Raf, EGFR, p-EGFR (Tyr1068), p-EGFR (Tyr1148), p-EGFR (Tyr1173), Her2, p-Her2 (Tyr1221/1222), PDK1, p-PDK1 (Ser241), mTor, p-mTor (Ser2448), HSP90, NF-kB, IKB, c-Kit, c-Kit (Tyr719), PDGFR, GSK3, beta-catenin, p-beta-catenin (Ser33/37/Thr41), stat3, p-stat3 (Ser727), stat5, p-stat5 (Tyr694), smad, p-smad (Ser463/465), CREB, p-CREB (Ser133)
Cell Growth/Cell Proliferation: Rb, P21, P27, P15, P16, P18, P19, CHK1, CHK2, DP-1, MDM2, BRCA1, BRCA2, GADD45, 14-3-3, Myt1, IL18,
Cell cycle: CDK2, CDK4, CDK6, CDC2p34, CDC25A, CDC25B, CDC25C, CyclinA, Cyclin B, Cyclin D, Cyclin E, Rb, CyclinA, Cyclin B, Cyclin D, Cyclin E, CDK7
DNA repair: 53BP1, Ape1, ATM, ATR, BLM, Brg1, CK1, CK2, REDD1, Claspin, ERCC1, ERCC2, P53, Rad1, Rad9, Rad50, Rad51, Rad52, Mre11, MSH2, XRCC1
Epithelial-to-mesenchymal transition/Adhesion: CD44, ICAM1, VCAM1, E-Cadherin, Collagen, ECM1, HAS1, Catenin, Integrin, Laminin, MMP1, MMP10, Selectin E, Selectin L, Thrombospondin,
Invasion/metastasis: uPA, uPAR, NF-kappaB, AP-1, MMP1, MMP2, MMP9, MMP13, CDH1, CDH2, E-cadherin, N-cadherin, ICAM-1, laminin-5, CD44, osteopontin, VEGF, Connexin 43 (Cx43), Slit2, Robo1, Cas, NM23, MKK4, CCR7, CXCR4
Transcription factor: AR, MYC, CEBF, DR1, E2F, EGR1, ELK1, ETS, FOXA2, FOXO1, ESR1, FOS, GATA, Jun, JunB, JunD, MYB, STAT, TBP, SMAD, PPAR, RB1, REL, E2F1, ER, PR

CONCLUSIONS

- Few studies have compared gene or protein expression in primary and metastatic SIC tumors resected simultaneously.
- Our findings suggest Protein Pathway Array reveal changes in a limited number of proteins, suggesting that these may be targets for therapy.
- Future studies are needed to validate these findings.

REFERENCES

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