CROSSTALK BETWEEN SOLUBLE FACTORS AND CELL-CELL INTERACTIONS: IMPLICATIONS FOR CELL CYCLE CONTROL AND TUMOR DEVELOPMENT

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ABSTRACT

Crosstalk between Soluble Factors and Cell-Cell Interactions:

Implications for Cell Cycle Control and Tumor Development

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Precise and dynamic control of cell behaviors, including proliferation, adhesion, and migration, is required for proper tissue organization and homeostasis. A key element to understanding how cellular functions are controlled lies in uncovering the topology of the molecular signaling networks that couple environmental signals to cellular responses. In this study, we have parsed the signaling networks involved in cell cycle regulation and tumor development and uncovered novel mechanisms of crosstalk between soluble factors and cell-cell interactions.

Our findings demonstrate that extracellular cues, including the epidermal growth factor (EGF), stimulate proliferative signaling through β-catenin, an intracellular protein that participates in both cell adhesion and transcription of cell cycle genes. In fact, EGF-mediated β-catenin transcriptional activity is an essential signal for proliferation of normal epithelial cells. Additionally, in a cancer cell system, we discover that EGF cooperates with Wnt 3a, a classical agonist of β-catenin transcriptional activity, to induce greater signaling than either ligand alone. Notably, EGF and Wnt 3a activate

transcription using different sub-cellular pools of β-catenin. Because hyperactive β-catenin signaling drives proliferation in cancer, this suggests that attenuation of β-catenin signaling may require different therapeutic strategies for EGF- and Wnt-driven tumors.

Since β-catenin signaling can be antagonized by sequestration with the cell-cell contact protein E-cadherin at the plasma membrane, proliferative signals mediated by β-catenin may regulate growth suppression at high cell density, a property of normal cells that is often lost during tumorigenesis. Indeed, in non-tumorigenic epithelial cells, we demonstrate that E-cadherin is upregulated in contexts where β-catenin signaling and DNA synthesis are suppressed. Additionally, exogenous E-cadherin suppresses proliferation with a strict requirement for β-catenin binding. Future studies to test the hypothesis that E-cadherin regulates the growth of normal cells will benefit from a quantitative assay developed to measure E-cadherin:β-catenin complexes. Such quantitative measurements are likely to be important because contact-mediated growth suppression by E-cadherin is coupled with a density-dependent, ligand-depletion mechanism that concomitantly regulates proliferation.

Finally, we demonstrate that EGF and other soluble factors synergistically control cell-cell interactions governing organization of normal epithelial cells into multicellular structures. Notably, this behavior resembles the program initiated during metastatic cancer, thus illustrating the flexibility of the epithelial phenotype even in non-cancerous cells. Together, these studies illustrate how the topology of molecular signaling networks can couple environmental cues including soluble extracellular factors and cell-cell interactions to regulate fundamental cellular functions.

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ABBREVIATIONS

Ab	Antibody
APC	*Adenomatous polyposis coli* gene product
BrdU	Bromodeoxyuridine
cAMP	Cyclic adenosine monophosphate
ChT	Cholera toxin
CREB	cAMP-responsive element-binding protein
DAPI	4',6-diamidino-2'-phenylindole-dihydrochloride
E-	Epithelial
EGF	Epidermal growth factor
EGFR	EGF receptor
EGTA	Ethylene glycol tetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EMT	Epithelial-mesenchymal transition
ERK	Extracellular signal-regulated kinase
Frz	Frizzled
GM	Growth medium
GSK3β	Glycogen synthase kinase 3β
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HGF	Hepatocyte growth factor
IgG	Immunoglobulin G
IP	Immunoprecipitation
Lef	Lymphoid enhancer factor
LRP	Low-density lipoprotein receptor
MOI	Multiplicity of infection
PAGE	Poly-acrylamide gel electrophoresis
pAkt	Phospho-serine 473 Akt
PBS	Phosphate-buffered saline
ppERK	Dually-phosphorylated (Thr202/Tyr204) ERK
PI3K	Phosphatidylinostitol 3-kinase

PCR	Polymerase chain reaction
PKA	cAMP-dependent protein kinase
PKC	Protein kinase C
pRb	Retinoblastoma protein
RTK	Receptor tyrosine kinase
RT-PCR	Reverse transcription PCR
shRNA	Short hairpin RNA
siRNA	Short interfering RNA
SDS	Sodium dodecyl sulfate
S.E.	Standard error
TBS	Tris-buffered saline
TBST	TBS plus 0.5% (v/v) Tween-20
Tcf	T-cell factor
TGFβ	Transforming growth factor β
VE	Vascular endothelial
VEGF	VE growth factor
VEGFR	VEGF receptor
v/v	Volume / volume