L#12 (December-6-2010)

Cancer & Signals
Hallmarks of Cancer
Therapy

Anti-cancer therapy must show *differential toxicity toward tumor cells* relative to normal cells.

Some unique properties of cancer cells not shared by normal cells, must be exploited.

In principle, cancer can be treated by inducing cancer cells to undergo

- Apoptosis
- Necrosis
- Senescence, or
- Differentiation.
Strategies

Disrupt cancer cell-autonomous processes, by ...

**Interfering** with autocrine/paracrine **signaling** within tumors,

**Blocking** heterotypic **signaling** between tumor cells and the surrounding stromal tissue or blood vessels

**Enhancing** immune **surveillance** against cancer cells expressing novel antigens
Addictions in Cancer

- “Oncogene addiction” (OA)
- “Tumor suppressor gene hypersensitivity (TSGH)”
- “Non-oncogene addiction” (NOA)
Theories of OA

• Oncogene addiction
  – Oncogenes elicit strong, opposing prosurvival and proapoptotic signals in cancer cells
  – Acute inhibition of oncogenes tilts this balance toward cell death

• To bring about their phenotypic manifestations, oncogenes rely on extensive adaptations in cellular processes that are themselves not oncogenic.

• In addition, cancer cells may also depend on the normal cellular functions of certain genes that act in oncogenic pathways but are not themselves classical oncogenes.
  – For example, mutations in many genes in a given oncogenic pathway are unable to directly promote tumor formation because, despite being required for their pathway, they cannot increase the overall activity of the pathway because they are not rate-limiting.
Non-Oncogene Addiction

• A reduction in the activity of many non-oncogenes in oncogenic pathway can become rate-limiting to the pathway in question, and thus, they represent potential drug targets.
  – By this rationale, cancer cells are addicted to both oncogenes and non-oncogenes.

• **Non-oncogene addiction, NOA**: The addiction of cancer cells to the functions of nononcogenes.
  – Although NOA genes, like oncogenes, are required for maintenance of the tumorigenic state, NOA genes do not undergo oncogenic mutations or functionally significant genomic alterations in tumors.
The Tumorigenic State

Diagram showing various factors contributing to the tumorigenic state, including:
- Restructured stroma
- Altered survival signals
- Hypoxic environment
- Altered metabolism
- Altered proliferation signals
- Genomic instability
- Aneuploidy and copy number variation
- Immune system evasion

Factors include:
- Dependence on heterotypic signaling
- Dependence on pro-survival signals
- Dependence on angiogenesis
- Glucose dependence TCA inhibition
- Oxidative stress
- DNA damage stress
- Mitotic stress
- Proteotoxic stress
- Novel epitope display

Connections to Tumor cell lethality include:
- Stromal signaling pathways
- Pro-survival pathways
- Angiogenic pathways
- Alternative metabolic pathways
- Stress support
- Immune suppression
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<th>Agent</th>
<th>Target</th>
<th>Addiction</th>
<th>Hallmarks</th>
<th>Potential mechanisms</th>
<th>References</th>
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<tr>
<td>17AAG (small molecule)</td>
<td>HSP90</td>
<td>NOA</td>
<td></td>
<td>A geldanamycin analog that binds to the ATP-binding pocket of HSP90 and inhibits its catalytic activity</td>
<td>Whitesell and Lindquist, 2005</td>
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<td>1MT, MTH-Trp (small molecule)</td>
<td>IDO</td>
<td>NOA</td>
<td></td>
<td>Inhibits tryptophan catabolism in tumor microenvironment to allow T cell proliferation</td>
<td>Muller and Scherle, 2006</td>
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<tr>
<td>5-fluorouracil (small molecule)</td>
<td>DNA</td>
<td>NOA</td>
<td></td>
<td>Inhibits pyrimidine metabolism, incorporation into DNA and RNA causes cell-cycle arrest</td>
<td>Longley et al., 2003</td>
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<tr>
<td>ABT-737, ABT-263 (small molecule)</td>
<td>BCL-XL, BCL-2</td>
<td>OA</td>
<td></td>
<td>Bind to the BH3 pocket of Bcl-XL and inhibit its antiapoptotic function</td>
<td>Stauffer, 2007</td>
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<tr>
<td>Alvocidib, PD 0332991 (small molecule)</td>
<td>CDKs</td>
<td>OA</td>
<td></td>
<td>Inhibit CDKs and induce cell-cycle arrest</td>
<td>Lee and Sicinski, 2006</td>
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<td>AP 12009 (antisense oligo)</td>
<td>TGFβ 2</td>
<td>NOA</td>
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<td>Inhibits tumor autocrine and paracrine signaling, reverses immune suppression in the tumor microenvironment</td>
<td>Muller and Scherle, 2006</td>
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<td>AZD2281, AG014699 (small molecule)</td>
<td>PARP1</td>
<td>NOA</td>
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<td>Inhibit base excision repair in homologous recombination repair-deficient cancer cells</td>
<td>Bryant et al., 2005; Farmer et al., 2005</td>
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<td>Bevacizumab (antibody)</td>
<td>VEGF</td>
<td>NOA</td>
<td></td>
<td>Inhibits endothelial cell recruitment and tumor vasculature</td>
<td>Folkman, 2007</td>
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<tr>
<td>BEZ235 (small molecule)</td>
<td>PI3K</td>
<td>OA</td>
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<td>Causes cell-cycle arrest in tumor cells and inhibits tumor angiogenesis</td>
<td>Maira et al., 2008</td>
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<tr>
<td>Bortezomib (small molecule)</td>
<td>Proteasome</td>
<td>NOA</td>
<td></td>
<td>Inhibits the catalytic activity of 26S proteasome and induces apoptosis</td>
<td>Roccaro et al., 2006</td>
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<tr>
<td>Drug</td>
<td>Target(s)</td>
<td>On/Off</td>
<td>Action</td>
<td>Reference</td>
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<tr>
<td>Celecoxib</td>
<td>COX2</td>
<td>NOA</td>
<td>Reverses immune suppression in the tumor microenvironment, inhibits tumor autocrine and paracrine signaling</td>
<td>Muller and Scherle, 2006</td>
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<td>Cisplatin and analogs</td>
<td>DNA</td>
<td>NOA</td>
<td>Induces DNA crosslinks</td>
<td>Siddik, 2003</td>
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<td>Erlotinib, Gefitinib</td>
<td>EGFR</td>
<td>OA</td>
<td>Inhibit EGFR tyrosine kinase by competing with ATP binding</td>
<td>Sharma et al., 2007</td>
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<td>GRN163L (modified oligo)</td>
<td>hTERT</td>
<td>OA</td>
<td>Mimics telomere sequence and inhibits the hTERT active site</td>
<td>Dikmen et al., 2005; Harley, 2008</td>
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<tr>
<td>GRNVAC1 (cell therapy)</td>
<td>hTERT</td>
<td>OA</td>
<td>Autologous dendritic cells transduced to express an hTERT-LAMP fusion protein to elicit T cell response to hTERT + tumor cells</td>
<td>Harley, 2008; Su et al., 2005</td>
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<tr>
<td>GV1001 (peptide)</td>
<td>hTERT</td>
<td>OA</td>
<td>A short immunogenic peptide from hTERT designed to elicit T cell response against hTERT + tumor cells</td>
<td>Harley, 2008; Nava-Parada and Emens, 2007</td>
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<tr>
<td>Imatinib, Dasatinib</td>
<td>BCR-ABL, c-Kit, Src, PDGFR, other TKs</td>
<td>OA</td>
<td>Tyrosine kinase inhibitor with multiple targets</td>
<td>Quintas-Cardama et al., 2007</td>
<td></td>
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<tr>
<td>Mapatumumab, Lexatumumab</td>
<td>TRAIL receptor</td>
<td>NOA</td>
<td>Bind and activate TRAIL receptors to induce apoptosis</td>
<td>Carlo-Stella et al., 2007</td>
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<tr>
<td>Methotrexate</td>
<td>DHFR</td>
<td>NOA</td>
<td>Inhibits thymidine biosynthesis and induces replicative stress</td>
<td>McGuire, 2003</td>
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<tr>
<td>Compound</td>
<td>Target</td>
<td>Activity</td>
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<td>Nutlin-3 (small molecule)</td>
<td>HDM2</td>
<td>Binds to HDM2 and inhibits the binding and ubiquitination of p53</td>
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<td>Oblimersen (antisense oligo)</td>
<td>BCL-2</td>
<td>Inhibits the expression of BCL-2 by blocking translation of its mRNA</td>
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<tr>
<td>Paclitaxel, Vinblastine (small molecule)</td>
<td>Mitotic spindle</td>
<td>Interferes with dynamics and stability of mitotic spindles, activate mitotic checkpoints, and induce chromosome mis-segregation</td>
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<td>PF-00477736 (small molecule)</td>
<td>Chk1</td>
<td>Prevents activation of the DNA damage response, leading to persistent DNA damage and replication stress</td>
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<tr>
<td>PRIMA-1, MIRA-1 (small molecule)</td>
<td>Mutant p53</td>
<td>Reactivate the function of mutant p53</td>
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<tr>
<td>Rapamycin, RAD001, Temsirolimus (small molecule)</td>
<td>mTOR</td>
<td>Inhibit protein synthesis</td>
<td></td>
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<td>Retinoic acid (small molecule)</td>
<td>RAR, RXR</td>
<td>Induces cellular differentiation</td>
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<tr>
<td>SAHBs (stapled peptide)</td>
<td>BCL-XL, BCL-2</td>
<td>Stapled BH3 domains that bind to BCL-2 family members and promote apoptosis</td>
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<tr>
<td>Sorafenib, Sunitinib (small molecule)</td>
<td>Multiple kinases (VEGFR, RAF, c-Kit, PDGFR)</td>
<td>Inhibit endothelial cell recruitment and tumor vasculature</td>
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<td>Topotecan, Irinotecan (small molecule)</td>
<td>Topo-isomerase I</td>
<td>Induce DNA breaks</td>
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<td>Trastuzumab (antibody)</td>
<td>ERBB2</td>
<td>Inhibits ERBB2 activation and induces immune destruction of cancer cells</td>
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Targets for Cancer Drug Development

• Only a subset of defective proteins may be effective target.
• “Small Molecules”... Low molecular weight organic compounds.
• Inhibit biochemical functions of
  – (i) TSGs (Tumor suppressor genes)/Gate-keepers
  – (ii) Repair Genes/ Care-takers
  – (iii) Oncogenes/ Developer
Oncoproteins

- Hyperactive oncoproteins have proven to be good targets:

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Drug</th>
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<tr>
<td>Anti-Erb8</td>
<td>Herceptin</td>
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<tr>
<td>Tyrosine-Kinase Inhibitor</td>
<td>ZD1839, 051-774</td>
</tr>
<tr>
<td>RAS Farnesyl-Transferase Inhibitor (FTI)</td>
<td>BMS 214622</td>
</tr>
<tr>
<td>RAF Inhibitor</td>
<td>BAY 43-9006</td>
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<tr>
<td>MEK Inhibitor</td>
<td>CI1040</td>
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<tr>
<td>mTOR Inhibitor</td>
<td>RAD 001</td>
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</tbody>
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Biochemistry of Proteins

• Small Molecules:
  – (i) Easy to synthesize
  – (ii) Easy to penetrate into the interstices of a tumor

• Target Molecules:
  – Must have strong and specific interactions with small drug molecules

*Protein molecule is considered **druggable** if it carries out an identifiable enzymatic function, and a well-defined catalytic cleft for this purpose...*
DRUGGABILITY

• Transcriptional Factors
  – Considered highly undruggable as they lack catalytic clefts (Protein-DNA interaction)
• Ras
  – Druggability is problematic. It has a catalytic function (e.g., GTPase function), but it is a negative regulator of Ras signaling
  – Similar issues with Tyrosine phosphatase which reverses the effect of tyrosine kinase
Druggable targets in protein-protein interactions

• Insert a small molecule between the two docking proteins
  – E.g., Cyclin-Cdk pair
  – MDM-p53
  – BH3-BCL2/BCL-X
  – B-catenin-CBP
Kinases are attractive druggable targets

- As oncoproteins, responsible for driving neoplastic proliferation
- As enzyme processing proteins, possess catalytic clefts
- 518 protein coding genes in the human genome; out of which 90 encode tyrosine kinases
Two approaches

• Rational Drug Design
• High Throughput Screening (HTS)
  – Relative effect of the drug on its intended target, compared to its off-target effect...
  – Herceptin
  – Gleevec
  – Iressa/Tarceva
Rapamycin

• mTOR = mammalian Target of Rapamycin – regulating circuit
  – Rapamycin binds directly to FKBP12; the dimer/complex associate with mTOR protein
  – mTOR normally phosphorylates two key governors of translation (p7056 kinase (S6K1) 4EBP1 & S6 proteins of the small ribosomal units
  – mTOR is also a key upstream activator of Akt/PKB (regulates apoptosis and proliferation)
mTOR
[End of Lecture #12]