The Role of Sensory Protein Kinases in the Metabolic Regulation of Cancer Cells

Julianne H. Grose
Metabolic Disease:
An Increasing Health Crisis
Resource Allocation: the Intracellular Fates of Glucose

- glucose
- Glycolysis
  - glucose-6-P
  - glucose-1-P
  - UDP-glucose
  - Glycolysis
  - Glucose metabolism
  - Pentose Phosphate Pathway
    - NADPH, vitamins, nucleotides, etc.
    - Pentose Phosphate Pathway
      - Membrane structural components
        - Glucans, etc.
    - Storage
      - Glycogen, etc.
    - Protein glycosylation
      - NADH, ATP, vitamins, amino acids
      - TCA cycle
        - Acetaldehyde
          - Ethanol
          - Fermentation
        - NAD
          - ATP
          - Respiration
Protein Kinases Regulate Cellular Processes Through Phosphorylation

Protein Kinases

ATP → ADP

Protein Kinases

Enzymatic Activity
Cellular Localization
Binding Partners
Stability/degradation
Human Protein Kinase Overview
Nutrient Sensing Protein Kinases

Nutrients
Amino acids

TOR

Cell proliferation
Gene transcription
Protein synthesis
Current Treatments with mTOR Inhibitors

The everolimus combined with aromatase inhibitor improved progression-free survival (10.6 vs 4.1 months) AND an increase in response rate (7% vs 0.4%).

*Current trials for glioblastoma, leukemia, sarcomas and colorectal, pancreatic, kidney, and lung cancers among others
In Breast Cancer, 50% of Tumors Harbor Somatic Mutations that Activate mTOR

<table>
<thead>
<tr>
<th>Drug</th>
<th>Class</th>
<th>Study Populations</th>
<th>Common Toxicities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Everolimus (RAD-001)</td>
<td>Allosteric mToR inhibitor</td>
<td>Breast cancer</td>
<td>Fatigue, stomatitis, diarrhea, rash</td>
</tr>
<tr>
<td>Temsirolimus</td>
<td>Allosteric mTOR inhibitor</td>
<td>Breast cancer</td>
<td>Fatigue, stomatitis, diarrhea, rash</td>
</tr>
<tr>
<td>Ridaforolimus</td>
<td>Allosteric mTOR inhibitor</td>
<td>Breast cancer</td>
<td>Fatigue, stomatitis, anorexia, diarrhea, nausea</td>
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<tr>
<td>AZ02014</td>
<td>mTOR (TORC1/2) kinase inhibitor</td>
<td>Breast cancer</td>
<td>Fatigue, stomatitis, anorexia, diarrhea, nausea</td>
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<tr>
<td>XL76S</td>
<td>Dual PI3-kinase/mTOR inhibitor</td>
<td>Breast cancer</td>
<td>Nausea, diarrhea, anorexia, rash elevated LFTS</td>
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<td>BEZ235</td>
<td>Dual PI3-kinase/mTOR inhibitor</td>
<td>Breast cancer</td>
<td>Fatigue, diarrhea, nausea, vomiting, anemia</td>
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<td>GDC-0980</td>
<td>Dual PI3-kinase/mTOR inhibitor</td>
<td>Breast cancer</td>
<td>Nausea, fatigue, diarrhea</td>
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</tbody>
</table>

FDA approved for advanced cancers
Why Might Protein Kinase Inhibitors/Activators Have Undesirable Side Effects??

Multiple and Overlapping Substrates

Hundreds Of Kinases With Conserved Catalytic Domain
Nutrient Sensing Protein Kinases

Nutrients
- Amino acids

AMP levels
(low ATP)

TOR
- Cell proliferation
- Gene transcription
- Protein synthesis

AMPK
- \( \uparrow \) ATP production
- \( \downarrow \) ATP utilization
AMPK Inhibits Growth & Promotes Energy Production

- Gluconeogenesis
- Fatty acid synthesis
- Fatty acid oxidation
- Cholesterol (isoprenoid) synthesis
- Glycogen synthesis
- Lipolysis
- Glycolysis

- Mitochondrial biogenesis
- GLU T4
- PGC1α, MEF2, NRF1
- PEPCK, G6Pase
- SREBP1c, HNF4α
- ACC1, ACC2
- HMGR
- GS
- TSC2
- CFTR
- eNOS, nNOS
- CD36, FAT
- GLUT1, GLUT4
- PFK2

- Protein synthesis
- Cell growth & protein synthesis
- Cl⁻/fluid secretion
- Blood flow
- Fatty acid uptake
- Glucose uptake

- AMPK regulates multiple pathways related to energy metabolism, growth, and protein synthesis.
Widespread Evidence That AMPK Activation Inhibits Cancer

<table>
<thead>
<tr>
<th>Cancer</th>
<th>AMPK activation affect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>Inhibits Invasion and metastasis</td>
<td>Cerezo M, 2013</td>
</tr>
<tr>
<td>Thyroid cancer</td>
<td>Increased remission rates</td>
<td>Rusavy Z, 2013</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>Growth inhibition</td>
<td>Karnev E, 2013</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Increased survival</td>
<td>Aksoy S., 2013</td>
</tr>
<tr>
<td>Uterin Serous Carcinoma (USC)</td>
<td>Inhibits proliferation and migration</td>
<td>Sarfstein, R. 2013</td>
</tr>
<tr>
<td>Prostate Cancer</td>
<td>Reduced mortality</td>
<td>Bensimon L, 2013</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>Inhibit proliferation</td>
<td>Rosilio C., 2013</td>
</tr>
<tr>
<td>Colorectal Cancer</td>
<td>Pro-apoptotic</td>
<td>Cho, S., 2013</td>
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</table>
A Pro-tumorigenic Role for AMPK

### Table 1. Summary of recent findings supporting the pro-tumorigenic role of AMPK

<table>
<thead>
<tr>
<th>Cell lines/animal models</th>
<th>Stress conditions (assays)</th>
<th>Major findings supporting pro-tumorigenic role of AMPK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Established model of glioblastoma development in the offspring of rats exposed prenatally to the mutagen N-ethyl-N-nitrosourea (ENU)</td>
<td>Solid tumor formation in vivo (brain tumor model induced by carcinogen)</td>
<td>AMPK is strongly activated during early stage of tumorigenesis in vivo.</td>
</tr>
<tr>
<td>LKB1-null MEFs transformed by oncogene</td>
<td>Anchorage-independent growth (soft agar)</td>
<td>LKB1-null MEFs are resistant to oncogene-induced transformation.</td>
</tr>
<tr>
<td>AMPK\alpha1\alpha2-double knockout MEFs transformed by H-Ras</td>
<td>Solid tumor formation in vivo (xenograft)</td>
<td>AMPK\alpha1\alpha2-double knockout MEFs are severely impaired in their ability to form tumors in vivo.</td>
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<tr>
<td>AMPK\alpha1\alpha2 double knockout MEFs transformed by H-Ras</td>
<td>Matrix detachment (cell viability)</td>
<td>AMPK confers anoikis resistance in transformed cells</td>
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<tr>
<td>Pancreatic cancer cell lines</td>
<td>Glucose deprivation (cell viability), anchorage-independent growth (soft agar) and solid tumor formation (xenograft)</td>
<td>AMPK knockdown using siRNA sensitizes cells to death during glucose deprivation and also impairs anchorage-independent growth and solid tumor formation.</td>
</tr>
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<td>Glioblastoma cell lines</td>
<td>Glucose deprivation (cell viability and spheroid migration)</td>
<td>AMPK signaling promotes cell survival and migration during glucose deprivation</td>
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<tr>
<td>Prostate cancer cell lines</td>
<td>Non-stress conditions (migration?)</td>
<td>CaMKK2 is increased by androgen and mediates androgen dependent regulation of cell migration through AMPK.</td>
</tr>
<tr>
<td>Prostate cancer cell lines</td>
<td>Non-stress conditions (cell proliferation and apoptosis)</td>
<td>Inhibition of AMPK by RNAi or compound C decreases cell proliferation and induces apoptosis.</td>
</tr>
<tr>
<td>Prostate cancer cell lines</td>
<td>Lipid deprivation (caspase activity and cell mass) and solid tumor formation (xenograft)</td>
<td>siRNA screening in breast cancer cell lines under lipid deprivation identified AMPK\beta1 subunit as an essential gene for survival.</td>
</tr>
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<td>Prostate cancer cell lines</td>
<td>Glucose deprivation (cell viability)</td>
<td>AMPK promotes prostate cancer cell survival during glucose deprivation.</td>
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<td>Hepatocarcinoma cells isolated from mouse primary tumor induced by myc, Akt, p53-/-</td>
<td>Solid tumor formation (orthotopic transplantation into liver capsule)</td>
<td>Myc-driven tumorigenesis requires AMPK activity that promotes mitochondrial metabolism.</td>
</tr>
<tr>
<td>Pancreatic cancer cell line and glioblastoma cell line</td>
<td>Matrix detachment (cell viability) and anchorage-independent growth (soft agar)</td>
<td>KSR2 promotes metabolic activity, anoikis resistance and anchorage-independent growth via AMPK.</td>
</tr>
</tbody>
</table>

The AMPK Pathway and Cancer

PAS Kinase: A Nutrient Sensing Protein Kinase

AMP levels (low ATP)

\[ \uparrow \text{ATP production} \]
\[ \downarrow \text{ATP utilization} \]

Nutrients
Mitochondrial signals
Growth factors
Stress

\[ \downarrow \]

Cell proliferation
Gene transcription
Protein synthesis

Nutrients (glucose)

\[ \downarrow \text{kinase} \]

Glucose metabolism
PAS kinase: an evolutionarily conserved protein kinase

**PAS domain alignment**

<table>
<thead>
<tr>
<th>hPASK</th>
<th>mmPASK</th>
<th>dmPASK</th>
<th>scPsK1</th>
<th>scPsK2</th>
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**Kinase domain alignment**

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</tbody>
</table>
PASK deletion: protection from HFD-induced obesity and liver triglyceride accumulation
PAS kinase-deficient mice protected from glucose intolerance and insulin resistance

Glucose tolerance

Insulin tolerance

Hao et al, PNAS (2007)
Mutations in hPASK Cause Hyperinsulin Secretion and areAssociated with Maturity Onset Diabetes (MODY)

Semplici et al, 2012