GMS 6644: Apoptosis

Drug Resistance and Apoptosis
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Outline of the Lecture

- Mechanisms of drug resistance
- Alterations in the apoptotic machinery in anticancer drug resistance
- Strategies for reversing drug resistance

*Suggested Reading:*
Drug Delivery

**Diffusion**
- E.g., vinblastine, doxorubicin

**Transport**
- E.g., nucleoside analogs

**Endocytosis**
- E.g., immunotoxins

Mechanisms of Drug Action

Cellular Mechanisms of Drug Resistance

Gottesman et al. (2002)
Mechanisms of Drug Resistance

- **Intrinsic**
  - Host factors
    - Decreased intracellular drug accumulation, due to poor absorption, rapid metabolism, or excretion of a drug
    - Inefficient delivery of a drug to its target (tumor cells)
    - Host-tumor environment
  - Genetic and epigenetic alternations
    - Activation of oncogens and inactivation of tumor suppress genes
      - Alternations in the cell cycle and checkpoints
      - Alternations in apoptotic pathways
Mechanisms of Drug Resistance (cont.)

- **Acquired** (Multidrug resistance – MDR)
  - Altered accumulation of drugs within cells
    - Increased drug efflux: expression of ATP-dependent efflux pumps (ABC transporters) such as P-glycoproteins (PgP) and related MDR genes
    - Reduced drug uptake: ineffective endocytosis
  - Induction of drug-detoxifying mechanisms
    - Increased DNA repair
    - Induction of cytochrome P450 mixed-function oxidases
  - Insensitivity to drug-induced apoptosis
### Chemotherapy Induced Resistance (CIR)

Resistance may be due to drug-induced mutations in cells.

Mutations may exist in the cell before drug treatment, and are further selected during treatment, leading to overgrowth of drug-resistant variants.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Primary (%)*</th>
<th>Relapsed (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etoposide</td>
<td>80</td>
<td>11</td>
</tr>
<tr>
<td>Ifosfamide</td>
<td>50</td>
<td>15</td>
</tr>
<tr>
<td>Teniposide</td>
<td>90</td>
<td>15</td>
</tr>
<tr>
<td>Vincristine</td>
<td>42</td>
<td>21</td>
</tr>
</tbody>
</table>

* Response rates to single-agent chemotherapy in patients with SCLC
Apoptosis and Anticancer Drug Resistance
Chemotherapeutic Agents

Cellular Damage

- Damage incompatible with survival
- Damage Sensors
  - Signal Transduction
    - Protective response
      - Heatshock response
      - Metallothionine induction
      - DNA repair
      - Cell cycle checkpoint activation
    - Apoptotic cell death

Cell death
Apoptosis-Resistance Phenotype

- The cross-resistance to different cytotoxic regiments in tumor cells because of failure of activation of caspases.
- Modulation of the efficiency of the coupling of drug-induced damage to the activation of apoptosis is a key mechanism of drug resistance.
- Defects in the activation of the caspase-3 proteolytic system upon treatment with chemotherapeutic compounds are associated with resistance to apoptosis.
- The lack of apoptosis correlates with failure to achieve complete remission.
Apoptotic Pathways

The mitochondrial pathway plays the central role in chemotherapy-induced apoptosis

- Mitochondrial cytochrome c release before or concurrent with caspase activation is observed in different cell types in response to drug treatments.
- Chemotherapeutic agents induce mitochondrial membrane disruption and mitochondrial release of cytochrome c that is inhabitable by Bcl-2 and Bcl-xL.
- Apaf-1 overexpression sensitizes cancer cells to chemotherapeutic agents, accompanied with increased caspase-9 and -3 activation.
- Cells deficient in Apaf-1 or caspase-9 are protected from apoptosis induced by anticancer drugs, whereas cells deficient in caspase-8 and -2 show no protecting effect against anticancer drugs.
Dysregulation of the Intrinsic Apoptotic Pathway in Cancer Cells

- **Upstream from the mitochondria**
  - Mutations on those targeting upstream components of the apoptotic program (*p53, PTEN, Akt, Ras*)

- **At the Mitochondria**
  - Bcl-2 family members (pro- and anti-apoptotic)

- **Downstream from the mitochondria**
  - Inhibitors of apoptosis proteins (IAPs) and heat shock proteins (Hsp70/90)
  - Epigenetic silencing of *Apaf-1, caspase-3* deletion, *etc.*

- **Caspase-independent mechanisms**
  - AIF (apoptosis inducing factor)
Quantitative and qualitative changes in factors composing the apoptotic machinery contribute to the sensitivity of cancer cells to chemotherapy.

The Balance between the pro-apoptotic signals engendered by the damage and survival signals presented in a cell determines the cellular fate.
Loss of p53 pathway function can contribute not only to aggressive tumor behavior but also to therapeutic resistance.
p53-Mediated Apoptosis

Models of p53 action:

- Transcriptional upregulation of pro-apoptotic genes
  - Pro-apoptotic Bcl-2 members
  - Death receptors (e.g. CD95 & DR5)

- Transcription-independent activation of Bax (BH3-like activity), initiating cyto c release.

Bratton & Cohen (2001)
p53 and Drug Resistance

- Loss of normal p53 function reduces drug-induced apoptosis and tumor regression.
  - p53 mutations
  - Defects in the p53 pathway
    Functional mutations or altered expression of its downstream effectors (PTEN, Bax, Bak, and Apaf-1) or upstream regulators (ATM, Chk2, Mdm2 and INK4a/ARF)

- In many tumor cells, pro-apoptotic signaling via BH3-only proteins is impaired, typically due to mutations in p53 (e.g., Bak, Bax, Puma, and Noxa)

- Effects of p53 on drug-induced apoptosis is determined by a variety of factors.

- Functional p53 does not appear to be a general determinant of anticancer drug activity in solid tumors.
Mitochondrial Death Pathways

Leist & Jaattela (2001)
Bcl-2 Family Proteins and Drug Resistance

- Bcl-2 promotes resistance to a wide range of anticancer agents and even prevent p53-independent deaths.
- Down-regulation of anti-apoptotic Bcl-2 members sensitize cells to chemotherapy.
- Post-translational modifications, e.g. phosphorylation of Bcl-2, protect cells from apoptosis induced by chemotherapeutic drugs.
- Anti-apoptotic Bcl-2 members are transcriptionally up-regulated in response to survival signals.
Inhibitors of Apoptosis Proteins (IAPs)

- Consisting of NAIP, XIAP, cIAP1, cIAP2, and survivin
- Suppress apoptosis by preventing procaspase activation and inhibiting the activity of mature caspases (caspase-3, -7, and -9) by directly binding to caspases.
- Expression of cIAP1/2 is stimulated by NF-κB-mediated survival signals.
- Negative regulators of IAPs: Smac/DIABLO, XAF1, and OMI/HTRA2
- Frequently overexpressed in cancer, and its downregulation induces apoptosis in chemoresistant tumors.
- Elevated survivin levels correlate with an adverse prognosis in many types of cancers.
Apoptotic and Survival Pathways Involving Bcl-2 Members

PI3K-Akt Survival Signaling

- Growth-factor regulated Ser/Thr kinase
- Frequently amplified in solid tumors
- Hyperactivation inhibits apoptosis to a range of apoptotic stimuli including anti-cancer drugs.

Vivanco & Sawyers (2002)
Regulation of cell survival by PKB/Akt
Strategies for Reversing Drug Resistance
Overcoming Drug Resistance

**Goals:** Maximize tumor cell killing while protecting normal cells from toxic side effects.

*Tumor-specific alterations in apoptotic programs provide opportunities to target cell death in a selective manner.*
Cancer Therapy: induction of apoptosis

- Targeting the core components of the cell-death machinery
  - Inhibition or bypass of resistant pathways
  - Reactivation of pro-apoptotic pathways

- Targeting proteins that modulate apoptosis, including protein kinases, phosphotases, heat shock proteins, transcriptional factors, and cell-surface receptors
Targeting of the apoptotic machinery

- Targeting anti-apoptotic activities
  - Modification of expression of Bcl-2 family members
    - Down-regulation of Bcl-2 expression
  - Cell survival signaling (NF-κB, EGFR, PI-3 kinase/Akt)

- Restoring p53-dependent pro-apoptotic activities
  - Reintroduction of wt p53 into p53 mutant tumor cells
  - Targeting mutated p53 to restore some p53-related transcriptional response (e.g., CP-31398)
  - Blocking interactions between p53 and its negative regulators such as MDM2 (e.g., nutlins)

Modulation of p53 as a stand-alone strategy is likely to be less effective than a strategy to enhance the efficacy of chemotherapy.
Targeting of the apoptotic machinery (cont.)

- Activation of p53-independent death mechanisms
  - Death receptor ligand–mediated cell death: preferentially inducing apoptosis in tumor cells (e.g. DR-4/TRIAL-R1 and DR-5/TRIAL-R2)
- Enhancing the effects of pro-apoptotic mutations

- Targeting of apoptosis regulators
  - Heat shock proteins (e.g. the PI3K-Akt pathway)
  - Proteasomes (e.g. the NF-κB pathway)
  - Protein kinases (e.g. PKC)
Modulating mitochondrial pathway by Bcl-2 family proteins
Cancer Therapeutics:
Killing Cancer Cells by Targeting Bcl-2-like proteins

- In many tumors, signaling via BH3-only protein is impaired due to p53 mutation.
- Nearly all tumors retain the core apoptotic machinery.
- Small molecules that supplant BH3 function should be highly effective anticancer drugs.
- ABT-737 binds like a BH3 domain to the groove of Bcl-X_L, which markedly enhances cellular response to chemotherapeutic drugs.

ABT-737 does not directly initiate the apoptotic process, but enhances the effects of death signals, displaying synergistic cytotoxicity with chemotherapeutics and radiation.

Oltersdorf et al. Nature 435: 677 (2005);
Regulation of p53-Mediated Death Pathway

Jones, Nature (2001)
Cancer Therapeutics: Inhibition of p53-MDM2 interaction

• ~50% of human cancers express wild-type p53, and its activation may offer a therapeutic benefit
• Overproduction of MDM2 as an alternative mechanism for disabling p53 function in tumors without p53 mutation
• MDM2 antagonists require not only wild-type p53 but also functional signaling in the p53 pathway
  – The apoptotic function of p53 is altered to varying extents, but the ability of p53 to induce cell-cycle arrest is well preserved
  – Cancer cells with mdm2 gene amplification are most sensitive to MDM2 antagonists (Tovar et al. PNAS 103:1888-1893, 2006)
Activation of the p53 Pathway by Small-Molecule Antagonists of MDM2, the Nutlins

Aberrant MDM2 expression enhances apoptotic cell death


Growth Factor Signaling in Cancer — Survival Signaling Pathways

Growth Factors

- Proliferation
- Differentiation
- Survival
Components of Ras-dependent Signaling Pathways Implicated in Human Cancer

Growth factor Receptor

Breast ca.

Colon ca.

Plasma Membrane

RAS

NF1 Schwannoma

Survival Motility Proliferation Differentiation

PI3-K

B-Raf

PTEN

TSC1/TSC2

Akt

mTOR

MAPK/ERK2

Breast ca.

Gastric ca.

Ovarian ca.

Prostate ca.
Apoptosis-inducing anticancer drugs in clinical trials

Summary

- Mutations in apoptotic programs arising during the course of tumor development (e.g. loss of p53 and overexpression of Bcl-2) can contribute to both intrinsic and acquired drug resistance.
- Activation of the molecular machinery of apoptosis is a convergence point for many cytotoxic agents, irrespective of the primary mechanism of drug action.
- Quantitative and qualitative changes of factors composing apoptotic pathways may relate to the sensitivity of cancer cells to chemotherapy.
- Novel therapies that target tumor-specific alternations in apoptotic pathways, either alone or in combination with conventional chemotherapeutic agents, may provide means to reverse drug resistance.