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CROSS RESISTANCE OF MELANOMA CELLS TO CHEMOTHERAPY AND TRAIL

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CELL KILLING MECHANISMS USED BY LYMPHOCYTES DEPEND ON INDUCTION OF APOPTOSIS

1. Granzyme – Perforin Mediated Killing
   CD8 CTL (CD4 CTL)
   NK Cells and ADCC

2. TRAIL (FasL, TNF-α) Mediated Killing
   CD4 T Cells
   Monocytes, Dendritic Cells
TRAIL Induces Apoptosis in the Majority of Melanoma Cell Lines
NEW CONCEPTS IN APOPTOSIS

TRAIL, Granzyme B

P53

Noxa, Puma
Bad

Bid

Bcl-2, Bcl-xL, Mcl-1

Bim, Bmf

Cytoskeleton

Bax, Bak

Mitochondria

Smac, Omi
Alternate Pathway

Cyto c, Casp 9
Classical Pathway

IAPs

Effector Caspases

3, 7
Prolonged exposure of melanoma cells to TRAIL results in TRAIL-resistant sub-lines
TRAIL-resistant melanoma cells are cross-resistant to various types of chemotherapeutic drugs
TRAIL-resistant melanoma cells are cross-resistant to various types of chemotherapeutic drugs
TRAIL-resistant melanoma cells are cross-resistant to FasL-induced apoptosis
AT LEAST THREE SIGNAL PATHWAYS INDUCE RESISTANCE TO TRAIL INDUCED APOPTOSIS
TRAIL Induces Rapid Erk1/2 Activation in Melanoma cells
U0126 Sensitises Melanoma to TRAIL-Induced Apoptosis
TRAIL Induces a Marked Increase in Reduction of the Mitochondrial Membrane Potential in the Presence of U0126
Increased Activation of ERK1/2 and Akt in TRAIL-Selected Resistant Cells

Mel-FH
Mel-FH.R
Mel-RM
Mel-RM.R

p-ERK1/2
ERK1/2
p-Akt
Akt
Inhibition of Akt signaling by the PI3-K inhibitor sensitizes melanoma to TRAIL-induced apoptosis
PKC Activation Differentially Regulates Sensitivity of Melanoma to TRAIL-Induced Apoptosis
Sensitization of Melanoma Cells to TRAIL by PMA Is Associated with Deficient PKCε Expression

PKCδ / PKCε

R2=6.4, p=0.0298

% Apoptosis by TRAIL

R2=0.94, p=0.0003

% Apoptosis by PMA+TRAIL
ARE THESE PATHWAYS INVOLVED IN CROSS RESISTANCE TO TRAIL AND CHEMOTHERAPY?
Inhibition of Akt, Erk1/2, or PKC does not sensitize TRAIL-resistant melanoma cells to TRAIL.
Inhibition of Akt, Erk1/2, or PKC does not sensitize TRAIL-resistant melanoma cells to TRAIL.
The expression levels of major anti-apoptotic Bcl-2 and IAP family members remain unaltered in TRAIL-resistant melanoma cells
Alterations in the expression levels of proapoptotic Bcl-2 family members in TRAIL-resistant melanoma cell lines

<table>
<thead>
<tr>
<th>Mel-FH</th>
<th>Mel-FHR</th>
<th>Mel-RM</th>
<th>Mel-RMR</th>
<th>MM200</th>
<th>MM200.R</th>
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</thead>
<tbody>
<tr>
<td><img src="Bad.png" alt="" /></td>
<td><img src="Bim.png" alt="" /></td>
<td><img src="PUMA.png" alt="" /></td>
<td><img src="Bak.png" alt="" /></td>
<td><img src="Bax.png" alt="" /></td>
<td><img src="Apaf-1.png" alt="" /></td>
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Apoptotic signaling induced by FasL and SBHA was inhibited upstream of mitochondria in TRAIL-resistant melanoma cells.
Decreased expression of p53 and p21, but not p27 in TRAIL-resistant cells
SUMMARY

- Vincristine, Cisplatin, SBHA show cross resistance with TRAIL
- Inhibition appears to be upstream of Mitochondria
- Activation of ERK and Akt pathways do not seem to be involved
- Selection of P53 variants possibly involved
TRAIL death receptor expression is down-regulated in TRAIL-resistant cells

![Graph showing TRAIL death receptor expression in parental and resistant cells](image)
The “Classical” Extrinsic Signaling Pathway Suggested for TRAIL-Induced Apoptosis

TRAIL → TRAIL-R1 or/and -R2 → FADD → Pro-caspase-8 → Activated caspase-8 → Bid → tBid → Caspase-3 activation → apoptosis

Apoptosome → Bax/Bak

Activated caspase-9
Decreased expression of PKCδ in TRAIL-resistant cells

- Mel-FH
- Mel-FH.R
- Mel-RM
- Mel-RM.R
- MM200
- MM200.R

PKCa
p-PKCa
PKCε
p-PKCε
PKCδ
p-PKCδ
GAPDH
Decreased expression of ICAD and PARP in TRAIL-resistant cells
The Alternative Intracellular Apoptotic Pathway Used by TRAIL in Melanoma Cells

- TRAIL
- TRAIL-R1 or/and -R2
- FADD
- Pro-caspase-8
- Activated caspase-8
- Bid
- tBid
- Caspase-3 activation
- apoptosis
- Bax/Bak
- Smac/DIABLO
- XIAP
TRAIL Is Involved in Killing of Melanoma Cells by CD4 T Cells

% Specific Cytotoxicity
% Apoptotic Cells

% Inhibition of Killing

mAB to TRAIL
mAB to FasL
mAB to TNFα

CD4 Clones

CD4 Clones
Effects of PKC on TRAIL-Induced Caspase-3 Activation, Cleavage of Its Substrates, and Release of Smac from Mitochondria

- Control
- TRAIL
- PMA + TRAIL
- GF109203X + TRAIL

Relative Cell Number

Fluorescent Intensity

Cleavage of PARP and ICAD

Release of Smac