

Aus der Klinik für Dermatologie und Allergologie  
der Medizinischen Fakultät der Charité – Universitätsmedizin Berlin

DISSERTATION

The mechanism of sensitivity and resistance of  
melanoma cells to tumor necrosis factor-related  
apoptosis-inducing ligand (TRAIL)

zur Erlangung des akademischen Grades  
Doctor medicinae (Dr. med.)

vorgelegt der Medizinischen Fakultät der Charité – Universitätsmedizin  
Berlin

von

Bahtier Kurbanov

aus Duschanbe. Tadschikistan

Gutachter: 1. PD Dr. rer.nat. Jürgen Eberle  
2. Prof. Dr. med. Martin Leverkus  
3. Prof. Dr. rer.nat. Christoph Hanski

Datum der Promotion: 21. November 2006  
Datum der Urkundenverleihung: 15. Dezember 2006

## CONTENT

<b>1. INTRODUCTION</b>	3
<b>1.1. Malignant Melanoma</b>	3
<b>1.1.1. Epidemiology</b>	3
<b>1.1.2. Melanoma progression</b>	4
<b>1.1.3. Clinical characteristic of early melanoma and classification</b>	4
<b>1.1.4. Prognostic parameters</b>	6
<b>1.1.5. Treatment of malignant melanoma</b>	7
<b>1.2. Apoptosis</b>	9
<b>1.2.1. Definition</b>	9
<b>1.2.2. Caspases as basal elements for proapoptotic pathways</b>	10
<b>1.2.3. Caspase inhibitors</b>	11
<b>1.3. Intrinsic (mitochondria) apoptotic pathways</b>	12
<b>1.3.1. The role of p53</b>	12
<b>1.4. Extrinsic apoptotic pathways</b>	13
<b>1.5. TRAIL and its receptors</b>	15
<b>1.5.1. TRAIL activates several pathways</b>	17
<b>1.5.2. Targeting tumors by TRAIL and its possible clinical application</b>	18
<b>1.5.3. Resistance to TRAIL</b>	23
<b>1.6. Nuclear Factor – kappa B (NF-κB)</b>	22
<b>1.7. Melanoma and TRAIL</b>	24
<b>1.8. Objective of the study</b>	26

<b>2. MATERIALS AND METHODS</b>	28
<b>2.1. Materials</b>	28
<b>2.1.1. Cell cultures</b>	28
<b>2.1.2. Cell culture media and solutions</b>	29
<b>2.1.3. Bacterial growth media and supplements</b>	29
<b>2.1.4. Antibiotics</b>	29
<b>2.1.5. Apoptosis stimulation agents</b>	30
<b>2.1.6. Enzymes</b>	30
<b>2.1.7. Expression plasmids</b>	30
<b>2.1.8. Kits</b>	31
<b>2.1.9. Molecular length markers</b>	31
<b>2.1.10. Extraction buffers for cellular proteins</b>	31
<b>2.1.11. Antibodies</b>	32
<b>2.1.12. Solutions</b>	33
<b>2.1.13. Chemical and radioactive substances</b>	34
<b>2.1.14. Equipment</b>	35
<b>2.2. Methods</b>	36
<b>2.2.1. Cultivation of cells</b>	36
<b>2.2.2. Freezing and thawing of cells</b>	36
<b>2.2.3. Detection of apoptosis</b>	36
<b>2.2.4. Cytotoxicity assay</b>	38
<b>2.2.5. Protein analysis</b>	39
<b>2.2.6. SDS-polyacrylamide gel electrophoresis</b>	40
<b>2.2.7. Western blotting</b>	43
<b>2.2.7. Immunodetection of blotted proteins</b>	45

<b>2.2.9. Proteasome inhibition and in vitro Viability Assay</b>	46
<b>2.2.10. Fluorescence-activated cell sorting (FACS) analyses</b>	47
<b>2.2.11. Determination of NF-κB activity</b>	49
<b>2.2.11.1. Determination of NF-κB activation by ELISA</b>	49
<b>2.2.11.2. Electromobility shift assay</b>	52
<b>2.2.12. Restriction analysis</b>	54
<b>2.2.13. Transient transfection</b>	56
<b>2.2.14. Immunohistochemistry and Immunocytochemistry</b>	56
<b>2.2.15. Statistics and general statements</b>	57
<b>3. RESULTS</b>	58
<b>3.1. Consistent expression of DR5 but selective expression of DR4 in melanoma cells lines</b>	58
<b>3.2. High sensitivity to TRAIL-mediated apoptosis in DR4-positive melanoma cell lines</b>	61
<b>3.3. Cytotoxicity follows apoptotic cell death after TRAIL treatment</b>	61
<b>3.4. Strong activation of apoptosis cascades in DR4-positive melanoma cells</b>	63
<b>3.5. Significance of the mitochondrial pathway for TRAIL-induced apoptosis in melanoma cells</b>	65
<b>3.6. Prevalence of DR4 for TRAIL-induced apoptosis in cell lines expressing both receptors</b>	68
<b>3.7. Significant expression of DR4 and DR5 in primary melanomas</b>	71
<b>3.8. DR4-positive melanoma cells reveal high NF-κB activation after TRAIL treatment</b>	73
<b>3.9. Parallel apoptosis induction and NF-κB activation by TRAIL in DR4-positive melanoma cells</b>	76
<b>3.10. Increased NF-κB activity by TRAIL is mediated thought DR4</b>	76
<b>3.11. No changes in the levels of anti-apoptotic proteins after TRAIL treatment but decrease of DR4 in SK-Mel-13</b>	78
<b>3.12. Transient resistance of DR4 melanoma cells selected with TRAIL</b>	79
<b>3.13. Reduced activation of NF-κB in TRAIL-resistant melanoma cells</b>	84

<b>3.14. TRAIL resistance of melanoma cells is related to downregulation of initiator caspases and DR4</b>	86
<b>3.15. TRAIL sensitivity can be restored by overexpression of initiator caspases and DR4 in selected cells and in resistant MeWo cells</b>	86
<b>4. DISCUSSION</b>	92
<b>4.1. High significance of DR4 expression in melanoma cells</b>	92
<b>4.1.1. Consistent expression of DR5 but limited of expressionDR4</b>	92
<b>4.1.2. High TRAIL-induced apoptosis in melanoma cells positive for DR4</b>	93
<b>4.1.3. Both TRAIL death receptors are expression in primary melanomas</b>	95
<b>4.1.4. Resistance to TRAIL-induced apoptosis caused by downregulation of initiator caspases and DR4</b>	96
<b>4.1.2. High TRAIL-induced NF-κB activation in DR4 expressing melanoma cells</b>	98
<b>4.2.2. High NF-κB is not the reason of upregulation of anti-apoptotic proteins</b>	98
<b>4.2.3. Resistance to TRAIL-induced apoptosis relates to downregulation of initiator caspases and DR4</b>	100
<b>5. SUMMARY</b>	103
<b>ZUSAMMENFASSUNG</b>	106
<b>6. REFERENCE LIST</b>	109
<b>7. LIST OF PUBLICATIONS</b>	123
<b>7.1. Original publications</b>	123
<b>7.2. Short publications/ Oral presentations / Posters</b>	123
<b>8. ABBREVIATIONS</b>	125
<b>CURRICULUM VITAE</b>	127
<b>Erklärung</b>	129
<b>Acknowledgement</b>	130