Mechanisms of TRAIL-resistance

Zhang, Baojie

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Chapter 7

Summary and Future Perspectives

Summary

TNF-related apoptosis-inducing ligand (TRAIL) has been shown to target tumor cells but not healthy cells *in vitro*. In addition, clinical studies have revealed that recombinant human TRAIL (Dulanermin) is well tolerated in patients. Taken together, the safety of treatment and targeted apoptosis in human bodies render TRAIL a promising anti-tumor therapeutic.

TRAIL is a member of the TNF superfamily and it is the only cytokine, which binds to two different death receptors, DR4 and DR5. Binding of TRAIL triggers the formation of DISC, which leads to caspase-dependent apoptosis. Besides inducing this apoptotic signaling pathway, TRAIL can activate non-canonical kinase pathways through the same death receptors. For instance, death receptors recruit other proteins to form a secondary complex instead of DISC upon the binding to TRAIL. This multiprotein complex initiates survival or proliferation signaling pathways. The ability of TRAIL to induce survival or proliferation is one of the reasons why tumor cells can become resistant to TRAIL[271]. In addition, the presence of death receptors on the plasma membrane is essential for initiating apoptosis. For instance, death receptors in autophagosomes act as decoy receptors binding to TRAIL for inducing autophagy in breast cancer cells[272].

In this thesis, we unraveled molecular mechanisms controlling TRAIL sensitivity in tumor cells using DR4- and DR5- specific TRAIL variants (**Chapter 2 and 3**). Moreover, we used combined treatment with epigenetic drugs to overcome TRAIL-resistance in tumor cells (**Chapter 5 and 6**).

Post-translational modifications, such as glycosylation, were found to correlate with sensitivity to TRAIL. In **Chapter 2**, we used agonistic receptor-specific TRAIL variants to dissect the contribution of FUT3 and FUT6-mediated fucosylation to TRAIL-induced apoptosis via its two death receptors, DR4 and DR5. We found that COLO 205 cells, which have a high-level of FUT3 or FUT6, are sensitive to both DR4 and DR5-mediated apoptosis. However, DLD-1 and HCT 116 cells, which show a relative low-level of FUT3 or FUT6, are only sensitive to DR4-mediated apoptosis. Therefore, we generated FUT3 or FUT6 overexpressed cell lines to investigate their sensitivities to TRAIL. Our data show that DR5-sensitivity is completely restored in FUT3 or FUT6 overexpressed cells. Furthermore, we revealed that fucosylation influences the formation of DISC and activation of caspase-8. Interestingly, we also observed that DR5-mediated apoptosis is improved by external administration of L-fucose.

Extracellular vesicles (EVs) are important in intercellular communication. EVs carry the messages including DNAs and proteins, from donor cells and deliver the contents to recipient cells. In **Chapter 3**, we firstly showed that conditioned medium (CM) derived from cancer cells

inhibits TRAIL-mediated cell death. In addition, we observed only DR5 but not DR4 in CM. Subsequently, we generated cell lines overexpressing long or short isoform of DR5 and proved that both isoforms contribute to the decreased number of apoptotic cells induced by TRAIL. Furthermore, we detected DR5 but not DR4 at the surface of EVs. Finally, we showed that TRAIL sensitivity is enhanced after depleting EVs from the medium.

Above two chapters provide new insights into understanding of TRAIL-resistant phenomena. Next, we focus on improving TRAIL sensitivity using combination strategies. Histones are the central components of nucleosomes, we therefore provide an overview of recent studies on the role of post-translational modifications of histones in **Chapter 4**. We also summarized strategies for combination therapy to improve TRAIL sensitivity by interfering with aberrant histone modifications using inhibitors.

Histone acetylation is one of the important modifications. This dynamic process is regulated by histone acetyltransferases (HATs), histone deacetylases (HDACs) and bromodomain proteins. In **Chapter 5**, we firstly used different HDAC inhibitors to investigate the alterations of TRAIL sensitivity on colon cancer cells. We found that RGFP966, a HDAC3-specific inhibitor, or PCI34051, a HDAC8-specific inhibitor, largely improve TRAIL sensitivity in combination with agonistic receptor-specific TRAIL variants. Furthermore, more apoptotic cells were observed upon the treatment with TRAIL variants in *HDAC1, 2,* or *3* downregulation cells. Finally, we proved that RGFP966 and PCI34051 improve TRAIL-induced apoptosis in 3D spheroid models.

Non-small-cell lung carcinoma (NSCLC) accounts for approximately 85% of cases of lung cancer. Clinical studies prove that EGFR-TKIs (EGFR-tyrosine kinase inhibitors) are more efficient therapeutics than chemotherapy. However, patients treated with the first generation of EGFR-TKI, such as erlotinib, can easily develop resistance. In **Chapter 6**, we combined a novel p300 and CBP-selective inhibitor A485 and TRAIL to overcome this problem. We showed that the A485-TRAIL combination synergistically increases cell death and decreases the volume of 3D spheroids of EGFR-TKI resistant cells. Furthermore, we proved that A485 augments TRAIL-induced apoptosis via the caspase cascade. This enhanced apoptosis is due to upregulation of gene expression of caspases, such as *CASP3, 7, 8* and *9*. Taken together, we demonstrate a successful combination of A485 and TRAIL in EGFR-TKI-sensitive and resistant NSCLC cells.

Future perspectives

The regulatory mechanisms for the TRAIL-induced apoptosis pathway are very complicated. However, binding of TRAIL to death receptors is the first step to initiate this

apoptotic signaling. Therefore, it is crucial to understand the molecular mechanisms on the regulations of this binding.

Firstly, the presence of death receptors on the plasma membrane is the basic requirement to bind to TRAIL. This binding to death receptors can be impaired by decoy receptors. We showed in Chapter 6 that DR5 displayed on EVs can act similar to a decoy receptor in preventing TRAIL to generate a signaling complex. However, it is still unclear on which type of vesicles DR5 is present. Two main groups of EVs are exosomes and microvesicles. The main differences between exosomes and microvesicles are their origins. In fact, exosomes are released intra luminal vesicles, which are generated from inward budding of late endosomes. While microvesicles are directly generated from outward budding of the plasma membrane. To investigate the presence of DR5 on vesicles, it is important to identify the markers during the processes of vesicle production. By unraveling the molecular mechanisms of secreting DR5 into extracellular space, future work should contribute to the understanding of DR5 trafficking.

In addition, post-translational modifications of death receptors are influencing TRAIL sensitivity. In Chapter 2, we showed that a lack of fucosylation contributes to the resistance to DR5-mediated apoptosis. Previous studies proved that N-glycosylation and O-glycosylation are also related to TRAIL sensitivity. However, the relationship of TRAIL sensitivity to other DR5 post-translational modifications, such as lipidation and ubiquitination, is still unclear. Moreover, the effect of the cross-talk between different modifications of death receptors on TRAIL sensitivity is not well understood.

At last, in Chapter 4 we provide potential targets for improving apoptosis-induction by TRAIL, such as PRMT5. It is interesting to test in future whether TRAIL sensitivity can be enhanced by inhibiting PRMT5 activity using small molecule inhibitors, such as JNJ-64619178 and GSK3326595.