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Vincent Piras
Keio University, Graduate School of Media and Governance

Project: Systems Biology Approach for the Understanding of Innate Immune Response and Cell Differentiation

Enhancing apoptosis in TRAIL-resistant cancer cells using fundamental response rules

The search to induce apoptosis, or programmed cell death, in cancer cells has led to the emergence of a new and fast growing field termed *cancer immunology*, also referred to as *tumor immunology*. Here, the interactions between the immune system with malignant cancers have shown the suppression of disease progression. Among the many immune factors found within the tumor microenvironment, the tumor necrosis factor (TNF) family members are noted for their ability to induce cellular apoptosis. In particular, the TNF-related apoptosis ligand (TRAIL), also known as Apo-2 ligand and TNFSF10, has received primal attention due to its ability to recognize and induce apoptosis of tumors and metastases while leaving normal cells mostly unaffected.

The endogenous TRAIL is prevalently found in several types of immune cells (e.g. macrophages, natural killer cells, T-cells) and its expression can be elevated in these cells by infected agents, such as, through the Toll-like receptor and the interferon gamma signaling pathways. TRAIL is known to bind with TRAIL-R1 (or death receptor (DR) 4), TRAIL-R2 (or DR5), TRAIL-R3 (or decoy receptor (DcR) 1), TRAIL-R4 (or DcR2) and osteoprotegerin. Notably, TRAIL-R1 and -R2 possess intracellular death domains and, subsequently, have the ability to mediate TRAIL-induced apoptosis. The remaining receptors are decoys that compete for TRAIL, thereby, possibly negatively regulate the effects of TRAIL-R1 and -R2 signaling.

The immune defense role of TRAIL was shown to kill pathogen-infected or malignant cells⁷. Notably, increased expressions of TRAIL-R1 and -R2 have been found on several kinds of tumor cells' extracellular membrane with corresponding increases in apoptosis compared with normal cells. The deficiency of TRAIL-R1 and -R2 has also led to malignancy. Further investigations using TRAIL-induced apoptosis for effective control of cancer proliferation have yielded successes at preclinical settings for certain cancer cells. In majority of cases, such as melanoma and neuroblastoma, however, TRAIL stimulation has little or no effect.

The non-sensitivity of TRAIL-stimulated cancers occurs due to several factors including: very low expression levels of TRAIL-R1 and -R2s, increased levels of DcR1, DcR2, elevated levels of negative regulators of apoptosis such as cFLIP, etc. On top of these, the upregulation of cell survival and proliferation pathways, through mitogen-activated protein kinases (MAPK) and nuclear factor-kB (NF-kB) activations, are crucial for the resistance.

Over the last decade, there has been great interest leading to numerous studies focusing on the usage of TRAIL, due to its ability to trigger the apoptotic pathways, as a strategy to fight the progression of cancer. Although successful in certain cancer types, TRAIL has not become a general candidate as many types of cancers are able to evade TRAIL's apoptotic property. Although recent works have shed light into the resistance mechanisms in TRAIL-based therapies, nevertheless, the understanding of counteracting cell survival and apoptotic pathways and finding ways to sensitize TRAIL-based strategy remain poor.

Drugs that upregulate TRAIL receptors (e.g. proteasome inhibitors) in resistant cancers may not be effective as they are likely to enhance both cell survival and apoptotic pathways with the net effect not necessarily enhanced cell death. Further studies on using combinatorial treatment of TRAIL with downstream targets that selectively suppress cell survival, such as NF-kB and MAP kinases inhibitors, or enhancing apoptosis by suppressing the suppressors of caspases have recently been investigated. The reduced cell survival activity or increased apoptosis produced, generally, an increase in the net effect of cell death, providing good prospective for increasing the efficacy of TRAIL-based strategies. However, these strategies have focused on suppressing either the cell survival or apoptosis activity, independently. It remains unclear which strategy among these is optimal for the various TRAIL-resistant cancer types and, hence, we require a strategy that considers dual mode suppressing the survival and enhancing the apoptosis pathway simultaneously.

Here, we report a systemic strategy that considers both the cell survival and apoptotic dynamics to provide a more mechanistic way to target TRAIL resistance. Our dynamic computational approach, successful

to model TLR and TNF pathways, is used to examine the signaling mechanisms of NF- κ B, MAP kinases and caspases activations in TRAIL-stimulated HT1080 cells. Starting from a literature curated generalized TRAIL signaling topology, firstly, using response rules we infer novel features, namely i) a FADD-independent pathway(s) to activate p38 and JNK, bypassing the primary and secondary DISCs and through novel molecules *Y* and *Z*, ii) a crosstalk between RIP1 and p38 via MKK3/6, iii) a crosstalk between p62 and the JNK pathway, and iv) intermediary step(s) or molecule(s) upstream of JNK. These inclusions are necessary for the computational model to successfully recapitulate experimental outcome in all investigated conditions (wildtype, RIP1 KD, FADD KD, caspase-8 KD, and TRAF2 KD).

Secondly, to determine the best strategy to induce apoptosis in TRAIL-resistant HT1080 cells, we investigated the net effect of NF- κ B, MAP kinases and caspases activations by evaluating their *cell survival metric*, *CSM*, and making a link to the survival ratios (*SRs*) for various KD conditions. Overall, our simulation data suggests that the optimal target is the novel molecule *Z*, whereby its removal is predicted to produce about 95% HT1080 cell population death.

Recent studies have indicated the roles of PI3K, Akt and MADD for TRAIL resistance. We believe that these may belong to the novel FADD-independent pathways, and one of these could well represent the molecule *Y*. On the other hand, the novel molecule *Z*, which is activated by p62 to specifically activate JNK in our model, acts like a connector between the primary and secondary DISC. Performing a search of the protein-protein interaction database for p62 interacting partners, we obtain protein kinase C (PKC) family members as likely candidates. Further literature search supports PKC-zeta as a possible candidate.

It is important to note that although our average response model may not pinpoint a specific target exactly, nevertheless, it will be worthwhile to investigate molecules that interact with p62 for the search for optimal target for effective cell death in TRAIL-resistant HT1080 cells. Taken as a whole, the systemic approach presented here provides a promising contribution towards systemically analyzing the dynamics of cell survival and apoptotic pathways, for the sensitization strategy for TRAIL-based cancer therapy.

In this paper, we show that novel features of the TRAIL signaling can be revealed through the law of conservation and first order response equations. From this result, we theoretically demonstrate that targeting a cell survival molecule at the survival and apoptosis pathway junction can provide an optimal solution to treat TRAIL-resistance. It suppresses survival activities and, at the same time, enhances apoptosis.

This result can be viewed surprising as the vast diversity of molecular constituents, issues of heterogeneity, spatio-temporal effects such as diffusion and crowding within cells, are likely to make the TRAIL signaling response non-linear and difficult to conceptualize computationally. In contrary, our data suggests that cells, as a population, are able to discard individual differences to achieve a global average response that follows simple rules. This is clearly the underlying success that our final first-order response model is able to simulate multiple experimental conditions.

Hence, although we do recognize that biological complexity such as heterogeneity and fluctuations or noises observed at single cell resolution are important, at the same time we do need to accept that biology, like any other complex system, possesses both microscopic (single cell) and macroscopic (population average cell) dynamics. Thus, it is necessary to treat the two dynamics distinct and investigate their individual merits. For example, stochastic fluctuations are necessary to induce probabilistic differentiation from genetically identical cells, allowing multi-cellular organisms to switch fates and states to yield diversity, such as for development or stress, which, otherwise, may be impossible from a purely deterministic system. On the other hand, the well-coordinated response of cell populations, such as differentiation or growth, demonstrates that the single cell noise could cancel out when ensembles of cells are formed to generate a stable and robust response. For instance, the observation of guided average behavior in the synchronization of neuronal signaling, persistence mechanisms of bacteria and collective decisions in ants are noteworthy.

In the future, as single cell techniques continue to make impressive progress, it will be interesting to compare the single cell dynamics of HT1080 cells in wildtype and PKC-z mutants with the population response presented here. Also, it will be crucial to investigate how the heterogeneous single cells responses in TRAIL signaling could be guided to provide a possible 100% cell death, at least in a dish. In this light, large-scale tumor sequencing data and the study of single cell noise will be critical to enhance our modeling aspects further to generate and investigate single cell response models.

Full text, figures and references: Vincent Piras, Kentaro Hayashi, Masaru Tomita and Kumar Selvarajoo. Enhancing apoptosis in TRAIL-resistant cancer cells using fundamental response rules. *Scientific Reports* 1:144 (2011)