From integrated signaling networks to high content microscopy: systems approaches for TT21C

Erik HJ Danen

Division of Toxicology, Leiden Academic Centre for Drug Research, Leiden University, Leiden, The Netherlands.

Toxicogenomics has played a major role in the past decade to uncover cellular stress responses that underlie chemical-induced adverse reactions at the cellular as well as organ level and subsequently apply transcriptomics-based classifiers to predict adverse outcome. The question remains what functional role these stress response pathways as well as the individual genes that underlie these stress responses play in the onset of adversity. We have integrated transcriptomics, phosphoproteomics, and RNA interference (RNAi) approaches and used time-resolved live cell high content imaging of cellular stress responses to identify critical cell signaling components that determine the breaking point from adaptation to cell stress versus maladaptation and onset of cell death.

In pluripotent stem cells, DNA damage triggers loss-of-pluripotency and apoptosis as a safeguard to exclude damaged DNA from the lineage. An intricate DNA damage response (DDR) signaling network ensures that the response is proportional to the severity of the damage. We combined an RNAi screen targeting all kinases, phosphatases, and transcription factors with global transcriptomics and phosphoproteomics to map the DDR in mouse embryonic stem cells treated with the DNA crosslinker cisplatin. Integrated networks derived from canonical pathways shared in all three datasets, were implicated in DNA damage repair, cell cycle and survival, and differentiation. Experimental probing of these networks identified, amongst others a novel, p53-independent mode of DNA damage-induced Wnt signaling that limits apoptosis. Our findings reveal a balance between p53-mediated elimination of stem cells, through loss-of-pluripotency and apoptosis, and Wnt signaling that attenuates this response to tune the outcome of the DDR. We currently explore several other newly identified signaling networks that modulate the outcome of the DDR.

To further reveal the complexity of dynamic toxicity-related signaling events we have developed systems microscopy technologies. Here, quantitative live cell confocal imaging of dynamic cell biology processes is followed by quantitative multiparameter image analysis to provide cell-to-cell dynamic data for systems biology modeling. A range of BAC-GFP reporters has been developed and expressed in HepG2 lines for this purpose. We have used this approach to study the complex signaling involved in drug-induced liver injury (DILI), an important clinical problem that involves crosstalk between drug toxicity and the immune system. Transcriptomics analysis established critical drug-induced toxicity pathways that act in synergy with the pro-inflammatory cytokine tumor necrosis factor α (TNFα) to cause cell death of liver HepG2 cells. Live cell imaging of oscillatory NF-kB cytoplasmic-nuclear translocations and activation of distinct gene reporters provided further insight into the joint regulation of these pathways by TNFα and compounds associated with DILI in humans. Focused RNAi experiments and pharmacological inhibition is currently employed to probe these pathways for critical hubs in liver cells that are targeted by drugs and pro-inflammatory cytokines and control life/death decisions.