DNA damage signaling networks: from stem cells to cancer

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Chapter 8

Summary and discussion

DDR in stem cells

Genomic instability is a threat for the normal proliferation and homeostasis of cells, and it can lead to cancer formation as well as the appearance of several types of degenerative and ageing disorders. The DNA receives large amounts of insults that come from endogenous metabolites of the cell as well as from external sources such as UV radiation or chemicals. In order to avoid malignant transformation and the transmission of harmed genomic material, cells have developed a DNA damage response (DDR). This response consists of a collection of pathways that collaborate to 1), sense and recognize the damage, 2) promote cell cycle arrest, and 3) to allow the repair of the damaged DNA or trigger apoptosis if the damage is too severe (Jackson et al. 2009). In cancer cells, this response is often altered, and several anti cancer drugs exploit this to specifically target cancer cells. Stem cells, which are multipotent cells that can give rise to almost every cell type, have to assure that their genomic material is intact when undergoing asymmetric division. Therefore, it is assumed that stem cells must have a strong DDR.

DNA repair mechanisms such as homologous recombination and mismatch repair are major repair pathways in stem cells whereas non-homologous endjoining, which is more error-prone is predominantly used by differentiated cells such as fibroblasts and somatic cells (Tichy et al. 2008 and 2010; Seita et al. 2010; Maynard et al. 2008). Embryonic stem (ES) cells, which can give rise to any cell lineage, are highly sensitive to DNA damage and undergo apoptosis when damage is not effectively repaired, which is triggered by the rapid accumulation of mutations due to their lack of G1 cell cycle checkpoint. However, since ES cells play a crucial role in the formation of the embryo, a function that is taken over by somatic stem cells in later developmental stages, loss of these cells due to DNA damage or deficient repair mechanisms, can induce premature aging as well as cancer (Hoeijmakers 2009). Therefore, stem cells have been increasingly related to cancer, hence cancer stem cells, which may provide the necessary pool of cells to certain types of tumors as well as confer resistance against cancer therapy (Visvader et al. 2008).

A recent publication has highlighted the differences in the DDR in tissue-specific and cancer stem cells, firstly noting the need of understanding the complexity of the DNA damage response, and secondly rising the interesting question whether cancer stem cells may exploit their enhanced DNA damage response to bypass and survive anti cancer therapies (Blanpain et al. 2011).

In this thesis I provide further insight into the complexity of the DDR. In addition to mechanistic understanding in stem cells the newly identified DDR components can be further studied in cancer cells and may lead to new clues for improved cancer therapy.

The role of p53 in ES cell DDR

Adult or somatic stem cells play a crucial role in the maintenance of tissue homeostasis in the adult body. In the early stage of the embryo, the ES cells, which are pluripotent (hence they can give rise to the majority of cell types),

are the ones responsible for providing the pool of cells to the developing embryo. Once the development enters later stages, the ES cells are lost, and adult stem cells, which are multipotent (meaning that they can give rise to a restricted number of cell types), are responsible to form tissues and maintain tissue homeostasis. The capability of these particular cells to give rise to several cell types forces them to have a very strict threshold when it comes to putative mutations in their genomes. Therefore, it has been reported that stem cells have a very strong DNA damage response that triggers repair or apoptosis in order to avoid the transmission of damaged genomic material.

Although, there is some debate about the role of p53 in DDR in ES cells (Zhao et al. 2010), the results in chapter 5 clearly demonstrate that p53 plays a major role in the DDR in mouse ES cells. In addition to up/down regulation of p53 target genes upon cisplatin exposure, we show that when p53 is silenced by either siRNA or shRNA, the ES cells are protected against cisplatin-induced apoptosis. In relation to cancer therapy, it is known that in many cancer types, p53 is mutated and therefore transformed cells become resistant to certain treatments (Jordan et al. 2000). Our results involving p53 in the DDR in ES cells are also linked to cancer stem cells, in which mutation of p53 can lead to tumor relapse or enhance of resistance to therapy (Jerry et al. 2008).

Thus, it is noteworthy that the role of the tumor suppressor p53, while extensively studied in many cell types, still needs to be clarified regarding its involvement in the DDR specifically in stem cells. Additionally, in order to assess p53 based cancer therapies as well as cancer stem cells targeted therapies, it is important to investigate cross talk between p53 signaling pathways and alternative DDR pathways affecting for instance differentiation.

Chemotherapy response - DDR and much more

In cancer cells, oncogenic signaling pathways affect sensitivity to chemotherapy. In addition, interactions with the microenvironment also determine how cells respond to genotoxic drugs. We demonstrate in chapter 7 that the expression of integrins, which anchor cells to the extracellular matrix (ECM), in combination with the expression of certain oncogenes, modulates sensitivity to the DNA damaging drug cisplatin. In particular, an oncogenic mutant of c-Src increases sensitivity to cisplatin only when β1 integrins are expressed. Another oncogene (e.g. Ras) or other types of integrins (e.g. β3) lead to cisplatin resistance. Surprsingly, increased sensitivity in Src-β1 cells is not mediated by the classical p53-mediated DDR, but rather by endoplasmic reticulum (ER) stress.

These findings indicate that besides the DDR, which is a complex and tightly orchestrated mechanism, other types of toxic stress can determine the response to chemotherapeutic drugs. Moreover, the outcome depends on the type of oncogenic lesions and interactions with the environment. Thus, although the understanding of the DDR is a crucial step for the discovery of new components and the design of new biomarkers and drugs, it must be noticed that cross talk with other signaling pathways makes it

extremely difficult to predict responses in different types of cancer.

DDR, an expanding network of signaling cascades

The systems biology approach described in chapter 5 has provided an overview of the crosstalk between pathways that might be involved in the DDR. Among them, we found the classical p53-mediated pathway, as well as other pathways not previously described to be involved in this particular response such as Retinoic Acid Receptor (RA) activation, TGFβ signaling and Wnt signaling. RA enhances the differentiation of ES cells (Schuldiner et al. 2000). In relation to DNA damage based cancer therapies, these target rapidly dividing cells, a characteristic of cancer cells. When ES cells enter a differentiation process, their cell cycle is slower (White et al. 2005), which might indicate that certain therapies would not affect their viability. In contrast, our study shows that once the mES cells enter differentiation by loss of AP staining, their sensitivity to cisplatin increases.

TGFβ signaling is believed to regulate differentiation and development of stem cells (Seuntjens et al. 2009; Pera et al. 2010) and it has been show to enhance chemo-sensitivity in certain types of cancer (Irigoyen et al. 2010). In our study, although a TGFβ signaling network was predicted, reporter assays did not substantiate this prediction and inhibitors and activators of TGFβ receptor signaling did not modify sensitivity. Wnt signaling has been extensively studied in relation to the stem cell biology, specially self-renewal, and also cancer (Reya et al. 2005). Wnt signaling has been also shown to maintain ES cells undifferentiated (Abu-Remaileh et al. 2010), a characteristic that has been hypothesized to be responsible for stem cells to be resistant to certain therapies. Indeed in our study, we are able to enhance Wnt activation using a GSK3β inhibitor, which results in increased cell viability against cisplatin. Although some publications show that Wnt signaling appears to be targeted by p53 (K.-H. Lee et al. 2010), we didn't find the same connection in our work, were p53 silencing does not affect TCF/LEF signaling.

All together this indicates that besides the classical DDR, partially mediated by p53, there is a clear involvement of pathways that cross-talk in response to genotoxic stress, which is of crucial importance to be noted when re-designing novel cancer therapies as well as discovery of new biomarkers.

The OMICS era, data generation vs data analysis and prediction: Systems Biology

It is obvious from the recent published scientific work that the amount of data generated has dramatically increased. This has been thanks to the appearance of rapidly developing technologies that allow the processing of large amount of samples and therefore the generation of larger data sets in a shorter amount of time such as sequencing, cDNA arrays, proteomics, phosphoproteomics, functional genomics, metabolomics and miRNA profiling (Chuang et al. 2010). In our main project, as described in chapter 5, we made use of several OMICS to unveil new components of the DNA damage response.

The clear advantage of using such

OMICS technologies is the amount of data that can be generated. In our case we generated three data sets: (i) Transcriptomics, which gave us a total of 2.269 differentially expressed genes (DEGs); (ii) functional genomics, where we initially silenced 2.351 genes; and (iii) phosphoproteomics, which revealed about 10.000 phosphorylation events in the particular context that we used in our approach. The disadvantage of such large datasets is that the analysis can become problematic and often can lead to confusion. The exploitation of data analysis software is crucial to start dismantling the generated data sets and therefore define what type of information is relevant or not according to the desired filters, which also demands background knowledge on the biology of the data sets (Pujol et al. 2010). Such software packages provide an initial pathway analysis as well as correlation between molecules and curated interactions and involvement in particular pathways, which can be used to predict the involvement of the molecules within the data set (Gehlenborg et al. 2010).

In our study described in chapter 5, we used the three data sets (functional genomics, transcriptomics and phosphoproteomics) to generate interaction-enriched networks to subsequently interrogate for common canonical pathways in which identified molecules where overrepresented. This methodology provided us with evidence pointing to novel pathways being activated in our ES cells in response to genotoxic stress. However, although this type of analysis was proven to be a powerful prediction tool, it was necessary to note that the analysis performed in silico was based on curated data from existing scientific literature and data bases, thus the results had to be taken as an indication of pathways where our molecules might be involved in, and they had to be subsequently confirmed by actual experimentation. Indeed, we could confirm the majority of the predictions given by the analysis of the data such as the involvement of p53 in the DDR in ES cells as well as the putative role of Retinoic Acid Receptor activation upon genotoxic stress, and importantly, new insights into Wnt signaling related to DNA damage in ES cells. However, we could not confirm the involvement of TGFβ in the DDR in our cellular system, which indicates that the prediction in silico might as well contain errors and limitations (Arrell et al. 2010). In order to improve the outcome of our in silico analysis, and therefore make the predictions more reliable, a further but rather technically challenging step could be taken, which is to integrate the OMICS data generated in our experimental procedures at a molecule-to-molecule level (T. Y. Kim et al. 2010; Tieri et al. 2011).

Data integration at this level might provide a better understanding of the correlation between the different biological processes that are used to generate the OMICS data sets e.g., relation between kinases found to be important to modulate the response to cisplatin and the molecules that are phosphorylated upon exposure to the drug.

DDR proteins identified in ES cells – relevance for cancer treatment

Cancer is a multifactor disease in which accumulation of mutations leads to cellular transformation which in turn provides cells with pathological advantages such as self-sufficiency in growth signals, insensitivity to anti-growth signals, tissue invasion and metastasis capabilities, limitless replicative potential, sustained angiogenesis and evasion of apoptosis (Hanahan et al. 2000). DNA damage is at the basis of such accumulation of mutations and formation of cancer (Hoeijmakers 2009). DNA damage is daily introduced into the cells by either exogenous or endogenous sources. In order to avoid accumulation of mutagenic DNA lesions, cells have developed a DNA damage response (DDR), which consists of a collection of pathways that involve sensing and recognition of the damage, repair and ultimately cell cycle arrest or apoptosis. Our work in chapter 4 reveals activation of non-classical pathways in response to DNA damage, such as MAPK signaling, cyclin and PKC signaling and cytoskeleton reorganization, which are elucidated by using a phosphoproteomics approach. In a wider approach, including phosphoproteomics, transcriptomics and functional genomics, the results described in chapter 5, also provide more insight into how cells prevent cancer formation by activation of alternative pathways that appear to be parallel to the classical DDR.

It will be crucial to validate hits in cancer cells to see if they are involved there as well. E.g., a sensitizer that also kills p53 mutant cancer cells would be a new drug target. New insights in the working mechanisms of these new components and pathways may provide novel strategies for the design of new biomarkers and new druggable targets as well as new cancer therapies.

However, as discussed in chapter 2, the biology of stem cells, which implies particular properties such as quiescence, enhanced drug transporters, high DNA damage repair capacity and decreased levels of reactive oxygen species; suggests that in the case of malignant transformation of these cells, they could contribute to cancer formation, hence cancer stem cells. The presence of cancer stem cells has been demonstrated in several types of cancer, and it has been shown that they can be responsible for the relapse of these cancers.

Although our studies haven't been focused on the presence of cancer stem cells and their biology, the fact that we used mouse embryonic stem cells as a model for DNA damage response as well as a chemotherapeutic drug to induce this particular damage, might provide insights that can be extrapolated to the cancer stem cell biology. In fact, a recent publication describing the differences between the DNA damage response in tissue-specific stem cells, together with the implications of this specificity regarding the cancer stem cell biology and cancer progression, demonstrates that the increasing knowledge about stem cell biology is of crucial interest in order to understand this complex response and to design novel therapeutic drugs, which might have to be driven to target cancer stem cells (Blanpain et al. 2011).

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