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Commentary

NRF2: The key to tumor- and patient-dependent chemosensitivity in biliary tract cancer?

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In their article in *EBioMedicine*, Ming Zhan and colleagues demonstrate that chemosensitivity of biliary tract cancer (BTC) could be predicted by combining information of genetic variability and expression pattern of proteins involved in the oxidative stress response [1].The insights of this study exemplify a central aspect of modern personalized theranostics: how can molecular pathological diagnostics improve the prediction of chemotherapy response with reliable precision? The master regulator protein of the interesting study of Ming Zhan and colleagues is the Nuclear factor-erythroid 2-related factor 2 (Nrf2) protein which is a key factor in the cellular defense mechanism against oxidative stress.

But, what is the link between oxidative stress, BTC chemosensitivity and Nrf2? Cellular homeostasis must be protected for multiple challenges and stress through the fluctuations in the nutrient/oxygen availability or by the presence of electrophiles or xenobiotics which lead to alterations in the redox balance. At high doses, the cellular response pattern subsequent to such stress include cell death due to damage of essential macromolecules such as lipids, proteins, and DNA, which are particularly susceptible to reactive oxygen species (ROS) [2,3]. Here, the Nrf2 signaling system comes on stage: Nrf2 acts as a cellular redox sensor and – by elevated ROS levels – is released from cytoplasmatic sequestration (by Kelch-like ECH-associated protein 1 (Keap1)) and promotes transcription of ROS-protective genes. Under conditions of low oxidative stress, Nrf2 is degraded by the ubiquitin-proteasome pathway, whereas oxidative stress stabilizes Nrf2 which in turn activates transcription of anti-oxidative proteins, enzymes involved in drug metabolization, and efflux transporters. As reviewed by Menegon et al., when aberrantly activated, Nrf2 tumor cells might experience survival advantages in terms of apoptosis inhibition, proliferation and chemoresistance [4]. The related mechanisms involved in the pro-oncogenic activation of the Nrf2/KEAP1 pathway are heterogeneous and include genetic and epigenetic alterations as well as transcriptional changes and interaction with regulation [5].

It was experimentally shown that inhibition of the nuclear import of NRF2 using CRISPR/Cas9 results in a reduced proliferation phenotype with a higher sensitivity to the chemotherapeutic agents cisplatin and carboplatin *in vitro* and in a xenograft mouse model [6]. In addition, recombinant NRF2-siRNA significantly reduced Nrf2-regulated ATP-binding cassette efflux transporters resulting in sensitization of human osteosarcoma 143B and MG63 cells to doxorubicin, cisplatin, and sorafenib [7]. Besides the ROS-based mechanism described below, Nrf2 might contribute to chemoresistance of cancer cells also by other ways including regulation of long noncoding RNAs or surface markers in specific cancer types [8,9]. In line with these results, the recent study of Ming Zhan et al. demonstrated that Nrf2 depletion by shRNA increased doxorubicin cytotoxicity by a ~2.5-fold shift of the IC50 value *in vitro* while over-expression of Nrf2 enhanced

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chemoresistance in BTC cells [1]. Similar results were found for tumor xenografts of BTC cell lines as illustrated by a slower growth rate of tumors expressing Nrf2-targeting shRNA and a more significant tumor inhibition by chemotherapy. In clinical BTC samples, increased Nrf2 expression was accompanied with poor prognosis for BTC patients receiving postoperative adjuvant chemotherapy. The authors further discovered three functional polymorphisms (*CAT*_rs769217, *GPX4*_rs4807542, and *GSR*_rs3779647) which are linked to the postoperative chemoresistance via GPX4, CAT, or GSR related expression levels. Knockdown of GPX4, CAT, or GSR proved the link between chemoresistance, elevation of ROS level and activation of Nrf2-ABCG2 pathway in BTC. Finally, the experimental data were summarized to an overall survival (OS) - related score which correlate to higher survival benefit from adjuvant chemotherapy compared with patients with low OS-related score. Taken together, Ming Zhan et al. demonstrated that (i) gene variants in *CAT, GPX4*, and *GSR* can be used as novel biomarkers for prediction of chemosensitivity in BTC, and, (ii) the Nrf2-mediated regulatory cascade might be a promising target for future optimization of the efficacy of chemotherapy in BTC patients [1]. However, as recently reviewed by Panieri and Telkoparan-Akillilar et al., the development of potent, specific and safe Nrf2 inhibitors still represent the critical step for further elaboration of Nrf2-based approaches to increase chemosensitivity [5,10].

Furthermore, a valid and effective approach also needs to take into account i) whether the concept of therapeutic interference with oxidative-stress related chemosensitivity is transferable to other tumor entities, ii) which tumor-specific effects and probably compensatory mechanism might be observed after treatment which anti-NRF2-drugs, and, iii) which interfering, non-canonical mechanisms of NRF2-activation might be relevant, especially in the context of combining drugs.

In summary, the presented data of Ming Zhan et al. provides strong evidence that gene polymorphism have an essential impact on oxidative stress-related genes, and, that these characteristics have a strong predictive value for the tumor's chemosensitivity and patient's prognosis. In this context, Nrf2 plays a central role which could be exploited for increasing chemosensitivity as part of an individualized concept for each tumor entity and patient [1]. Combining all features of oxidative stress-related chemosensitivity, this approach is highly interesting, since it sheds light into the black-box of chemosensitivity and opens a door for new therapeutic options – which is especially important for devastating tumor entities that frequently develop chemoresistance such as BTC.

Declaration of Competing Interest

None.

CRediT authorship contribution statement

Tobias Kiesslich: Writing - original draft, Writing - review & editing. **Christian Mayr:** Data curation, Writing - review & editing. **Daniel Neureiter:** Writing - original draft, Writing - review & editing.

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