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AUTHOR'S VIEWS

A perspective on DNA damage-induced potentiation of the pentose phosphate shunt and reductive stress in chemoresistance

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ABSTRACT

Metabolic rearrangements and genome instability are two hallmarks of cancer. Recent evidence from our laboratory demonstrates that persistent DNA lesions hampering transcription may cause glucose rerouting through the pentose phosphate shunt and reductive stress. Here, we highlight the relevance of these findings for cancer and chemoresistance development.

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Cancer cells display distinctive biological features – for instance, extremely high rates of nucleic acid synthesis – that may be sustainable only under certain specific metabolic conditions. Consistently, alterations in metabolism are hallmarks of cancer, as notably exemplified by the Warburg effect. Genome instability is another distinctive feature of cancer; increased DNA damage burden and defects in DNA repair are fundamental causative elements in carcinogenesis, underlie the extreme clonal variability in tumors, and are major determinant of chemoresistance.

Genome instability and metabolic alterations are intertwisted characteristics of cancer and evidence principally gained at transcriptional level revealed differences in the metabolic layout of organisms with defective DNA repair. Changes in mRNA levels, however, are not sufficient to provide an accurate depiction of the metabolic landscape, which is largely modulated by allosteric regulation, independently from both transcription and translation.

In a recent study, we characterized metabolic rearrangements occurring in mouse models and patients' specimen with impaired transcription-coupled- and global-genome-nucleotide excision repair (TC-NER and GG-NER, respectively). Here, we described a mechanism connecting transcription stalling caused by defective DNA repair with augmented intracellular ATP levels, which in turn allosterically inhibit the glycolytic enzyme ATP-dependent 6-phosphofructokinase (*Pfk*, best known as phosphofructokinase) to reroute glucose through the pentose phosphate pathway (PPP). Potentiation of the PPP is intrinsically associated with increased production of NADPH reducing equivalents – which are generated in the oxidative branch of the pathway – that in our experimental system is not paralleled by proportionate production of oxidant species and/or endogenous oxidoreductase activity, and therefore culminates in reductive stress[1] (Figure 1A).

GG-NER defects cause cancer and imperfect TC-NER promotes aging – i.e. the major risk factor for cancer; moreover, chemicals inducing NER amended DNA lesions that also block transcription are currently used in clinical practice as chemotherapeutic drugs. Our findings may therefore be of particular relevance in the processes of carcinogenesis, tumor growth, and chemoresistance (Figure 1B).

Activation of the PPP may promote survival of cancer cells.[2] In rapidly dividing cells, the vast majority of pentose phosphate sugars and ribonucleotides incorporated in DNA are products of the PPP, as demonstrated by elegant ^{13}C-tracing experiments.[3] PPP potentiation may therefore represent an undesired effect of DNA-damage-inducing therapies leading to higher bioavailability of nucleic acids, which could help sustaining fast replication in resistant clones. In line with this concept, multiple factors important for carcinogenesis and/or chemoresistance – including, but not limited to suppression of tumor protein p53 (TP53, best known as p53) and activation of ataxia telangiectasia mutated (ATM)[4,5] – positively regulate the PPP. Moreover, proper function of the PPP is essential for cancer proliferation, at least in some cases, because inactive 6-phoshogluconate dehydrogenase (6PGD) – which is part of the PPP oxidative branch – causes senescence in lung carcinoma.[6] This effect is partially mediated by reduced redox buffering capacity and by increased oxidant stress, which are known causes of senescence.

According to our model, DNA-damage-induced transcription block is not *per se* sufficient to activate the PPP and also requires high ATP levels. In our experimental paradigm – i.e. defective TC- and GG-NER caused by a truncation in the excision repair cross complementation group 1 gene (*Ercc1)* – augmented intracellular ATP concentration is caused by a decline in macromolecular synthesis, i.e. a highly demanding process from the bioenergetics standpoint. Because cancer cells are characterized by extreme rates of DNA synthesis, one could argue that ATP surplus is unlikely to occur under these circumstances. In cancer, however, there are cases that could conceivably be associated with macromolecular synthesis reduction. For instance, at

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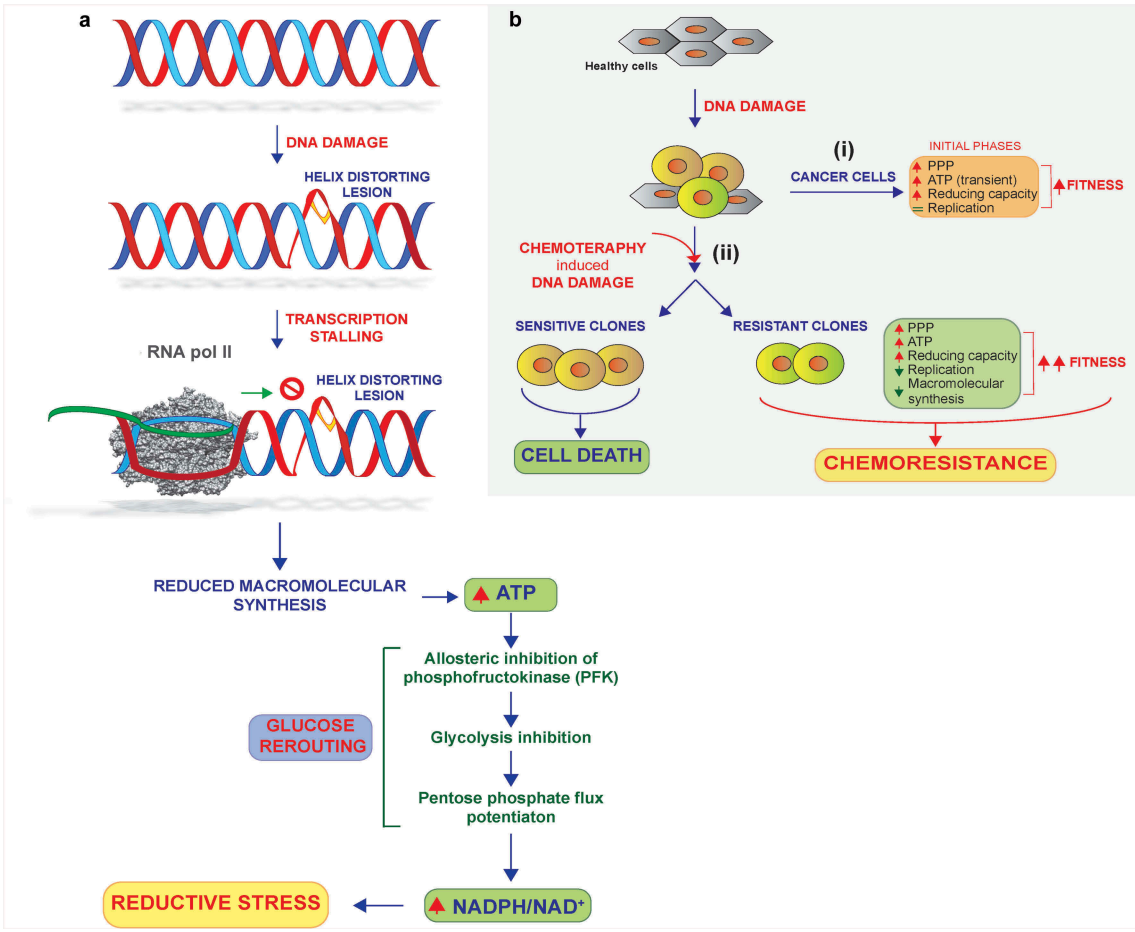

Figure 1. Metabolic rewiring caused by transcription-stalling DNA damage and its contribution to tumor chemoresistance. (A) Cascade of events leading to reductive stress upon accumulation of transcription-blocking DNA damage that causes stalling of the multi-protein complex RNA polymerase II (RNA pol II), reduced transcription output, and thus decreased macromolecular synthesis. These alterations culminate in augmented concentrations of ATP that inhibit the glycolytic enzyme ATP-dependent 6-phosphofructokinase (PFK, best known as phosphofructokinase) via allosteric mechanisms. Glucose is therefore diverted through the pentose phosphate pathway (PPP), with consequent increased production of NADPH that increases the reductive capacity of the cell. In the absence of commensurate pro-oxidant factors, the latter culminates in reductive stress (RNA pol II structure PDB code: 1i6h). (B) DNA-damage induces potentiation of the PPP and reductive stress, which may contribute to carcinogenesis and chemoresistance. (i) DNA damage could lead to the development of cancer cells via lesions that also induce transcription stalling. In cases where the DNA damage burden is not yet sufficient to miss checkpoints, and therefore if replication is not yet rampant, transcription stalling may lead to transient increased ATP concentration, glucose rerouting through the PPP, and reductive stress, which could provide these clones with increased fitness. Comparable phenomena may occur during chemotherapy; (ii) resistant clones, which may exhibit lower replication rates, use transient ATP surplus to reroute glucose through the PPP, to increase cellular reductive capacity, and to improve to clonal fitness. These metabolic rearrangements may ultimately favor chemoresistance.

very initial stages of carcinogenesis, when replication is not yet rampant, transcription stalling DNA damage caused by intrinsic genome instability may be associated with ATP level sufficient to inhibit glycolysis. A further possibility stems from recent evidence supporting the concept that development of chemoresistance may parallel development of antibiotic resistance in bacteria.[7] Here, in initial phases, growth – and thus macromolecular synthesis – is highly reduced in resistant cells. These circumstances may promote transient high levels of ATP that could temporarily potentiate the PPP and oxidant defenses in those clones that will resist treatment. Consistently, recent studies based on ultra-short ^{13}C tracing experiments indicate that glucose rerouting through the PPP represents an immediate and necessary response to oxidant-stress in skin fibroblasts and suggest that PPP activation may participate in development of resistance to therapies based on stimulation of toxic reactive oxygen species (ROS) production.[8] Intervening on these processes to halt PPP potentiation may therefore offer interesting therapeutic perspectives to improve current chemotherapy approaches.

Our study reveals that glucose rerouting through the PPP in TC-NER and GG-NER defective specimens culminates in reductive stress. The latter deserves special mention because – differently than oxidative stress – it has not received adequate investigative attention. Thus, despite unambiguous evidence demonstrating that excessive reducing capacity is detrimental, our understanding of reductive stress is still highly rudimentary. It is only very recently that redox biology has been approached more holistically – beyond the traditional oxidative stress concept – recognizing the importance of alterations in redox couples caused not only by excess of oxidants, but also by a reducing equivalent surplus.[9] While further investigative efforts are required to characterize the biological impact of reductive stress, some consequences may be envisaged to be very relevant for

cancer. For instance, reduction in the NAD(P)H/NAD(P)$^+$ redox couple – similarly to what we detected upon persistent transcription stalling – occurs also during hypoxia,[10] which is a major complication of cancer that severely aggravates prognosis. It is tempting to hypothesize that – during DNA damage-based chemotherapy – persistent transcription stalling in slow-growing, potentially resistant clones may cause a detrimental metabolic phenotype that parallels hypoxia.

Overall, we believe that our findings provide novel hints on the possible consequences of DNA-repair-driven metabolic redesign on cancer. Obviously, further studies are warranted to verify the relevance of our model for cancer and to test whether interventions targeting glucose rerouting, PPP activation, and excessive reductive capacity may constitute amenable strategies to treat cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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