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RESEARCH HIGHLIGHT

Spontaneous DNA damage propels tumorigenicity

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High levels of endogenously generated DNA damage drive oncogenesis, sustain malignant progression and increase therapy resistance. In a paper recently published in *Cell Research*, Liu and colleagues added additional insights into this topic by uncovering a novel intrinsic source of double-strand breaks that fosters the aggressiveness and stemness of malignant cells.

DNA double-strand breaks (DSBs) are a type of DNA damage characterized by the severance of both strands of the DNA duplex. Besides being produced by exogenous genotoxins, these cytotoxic lesions can arise from multiple endogenous sources, including (1) metabolic by-products, mostly reactive oxygen species (ROS); (2) prolonged stalling in DNA replication fork progression as this occurs during replication stress (RS); (3) chromosome missegregation events; (4) telomere shortening; and (5) chromothripsis, a phenomenon associated with the formation of micronuclei [1-3]. Endogenous DSBs are boosted by features associated with (pre)malignancy, such as increased proliferation rate, metabolic rewiring, activated oncogenes, deficiencies in DNA damage response (DDR), karyotypic aberration(s) or chromosomal instability (CIN) [4, 5].

Irrespective of their origin, DSBs are quickly detected by the ATM serine/threonine kinase (ATM). Upon binding to DSBs, ATM activates one or more branches of DDR by phosphorylating factors involved in DNA damage repair, cell cycle checkpoint and/or regulated cell death, such as the checkpoint kinase 2 (CHEK2/CHK2), tumor protein p53 (TP53/p53) and the histone variant H2A histone family member X (H2AFX/H2AX). This multi-pronged cascade also comprises auto-activation steps, feedback loops and a close interplay, and possibly redundancy, with the ATR serine/threonine kinase (ATR) network [6].

A wide range of evidence demonstrates that unrepaired DSBs can promote tumorigenesis by inducing DNA mutation(s) and karytotypic aberration(s) [7]. Moreover, established cancer cells often show increased DSB formation accompanied by an extensive DDR rewiring, encompassing defects in pathway(s) for DNA repair and/or the overactivation of the ATM and ATR axes [4]. This holds true also for cancer stem cells (CSCs) [8], which are the subset of immature, self-renewing and multipotent cells within the tumor mass driving cancer initiation and evolution [9]. Logically, DDR-related molecules are hence regarded as candidate targets for effective anticancer therapies [4].

In a recent paper published by *Cell Research*, the group of Chuan-Yuan Li reports an alternative, intriguing point of view about the source of endogenously induced DSBs and their contribution to cancer evolution [10]. When performing a comparative analysis of a panel of malignant, immortalized/untransformed and primary cell lines previously synchronized in the G_1 phase, these authors found high levels of endogenously induced DSBs exclusively in tumor cells. The presence of DSBs was assessed by quantifying foci containing γH2AX and tumor protein p53 binding protein 1 (TP53BP1/53BP1) and by the COMET assay. These lesions, which were dubbed as "self-inflicted" or spontaneous DSBs (spDSBs), originated in viable cancer cells independently of ROS and RS (as they occurred before S phase entry) through the order of events involving: (1) the partial permeabilization of the outer mitochondrial membrane, (2) the release of cytochrome c1 (CYC1) from mitochondria, (3) sublethal activation of executioner caspases (caspase 3 (CASP3), CASP6 and CASP7), and (4) cleavage of chromatin DNA by the apoptotic nucleases endonuclease G (ENDOG) and DNA fragmentation factor β (DFFB/CAD) (Figure 1). This circuitry was elegantly unveiled by CRISPR/Cas9 gene editing-mediated knockout and epistatic experiments revealing limited nuclease activation in *CASP3*$^{-/-}$, *CASP3*$^{-/-}$*CASP6*$^{-/-}$ and *CASP3*$^{-/-}$*CASP6*$^{-/-}$*CASP7*$^{-/-}$ cancer cells, as well as a significant decrease of spDSBs in tumor cells deficient for executioner caspases, *ENDOG* or *CAD* [10]. This mechanism is reminiscent to that observed upon exogenous perturbations [11], even though it occurred in unperturbed conditions.

In subsequent experiments, Liu *et al*. provided evidence in favor of the role of spDSBs in sustaining cancer cell tumorigenicity and stemness potential. They demonstrated that the subpopulation of cancer cells with elevated levels of spDSBs (spDSBs^{high}) displayed higher clonogenicity in soft-agar assay and grew more efficiently when xenografted in nude mice than the spDSBs^{low} fraction. In line with this evidence, strategies aimed at minimizing spDSBs (e.g., by knocking out effector caspases or knocking down pro-apoptotic BCL2 proteins) and boosting spDSBs (e.g.,

by irradiating spDSBs^{low} cells with low doses of x-rays) dwindled and enhanced the *in vitro* clonogenicity and *in vivo* tumorigenicity of malignant cells, respectively [10].

The increase in tumor aggressiveness was associated with the constitutive phosphorylation/activation of ATM. Activated ATM ultimately ignited the pro-tumorigenic nuclear factor-kappaB (NF-κB) and signal transducer and activator of transcription 3 (STAT3) pathways instead of triggering DDR. Thus, knockout of executioner caspases, *ENDOG* or *CAD*, reduced the level of phosphorylated ATM (pATM) and STAT3 (pSTAT3), and abrogated NF-κB activation. In addition, cells deficient for *ATM* did not constitutively activate the NF-κB/STAT3 axis, and displayed reduced soft-agar colony-forming ability and *in vivo* tumor growth [10]. In these experimental settings, knockout of *ATR* abolished the clonogenic and tumorigenic potential of *ATM*$^{-/-}$ cancer cells, confirming the functional interrelationship between these DDR kinases that has previously been reported [6].

Finally, taking advantage of patient-derived glioma cells, Liu and colleagues demonstrated that the subpopulation expressing the CSC marker prominin 1 (PROM1/CD133) displayed higher levels of spDSBs, pATM and pSTAT3 than the CD133-negative subpopulation. By employing CRISPR/Cas9 technology on these patient-derived glioma cells, Liu *et al*. demonstrated that *ATM* knockout decreased the CSC fraction. This was accompanied by the downregulation of STAT3 and resulted in diminished sphere-growing ability, clonogenicity and *in vivo* tumor growth [10]. In these experimental settings, the forced expression of constitutively active STAT3 rescued the stemness and tumorigenic potential of patient-derived glioma cells.

Reportedly, CSCs of distinct tissue origins constitutively activate DDR to resist genotoxic perturbations [8]. In this context, our own observations indicate that a subset of CSCs derived from colorectal cancer patients display ongoing RS response, at baseline, including the overactivation of ATM, associated with *TP53* mutations and increased chromosomal content [12]. It will be interesting to analyze (1) whether the *TP53* status and/or aberrant karyotype play a role in spDSB generation, (2) what is the mechanism preventing DDR execution upon spDSB-mediated ATM activation, and (3) whether a threshold level of spDSB tolerability exists and, if so, might be exploited for therapeutic purposes.

Importantly, the study of Liu and coauthors support the development of biomarker-driven anti-cancer strategies based on the inhibition of the ATM or ATR axis to deplete aggressive cancer (stem) cells bearing high levels of spDSBs and overactivated ATM/ATR. To fully translate this evidence into the clinics, it appears urgent to identify specific markers of spDSBs and to elucidate the precise origin of spDSBs, and the mechanisms and consequences of their formation during tumorigenesis.

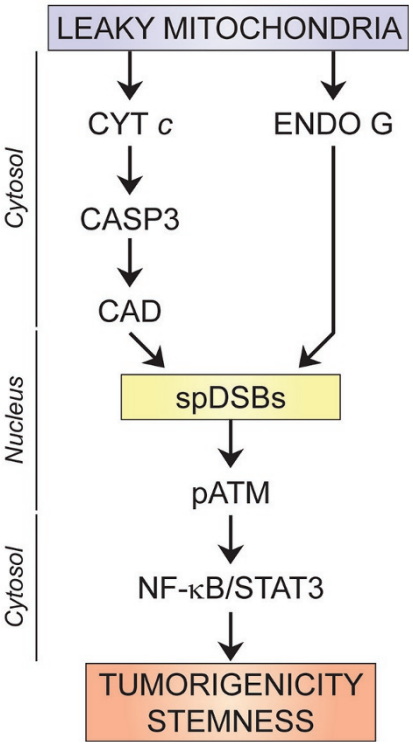

Figure 1 Mechanism and consequences of spDSB formation in tumors. spDSBs in cancer cells are generated via a mechanism involving the release from mitochondria of sublethal amounts of ENDOG and CYC1, which in turn unleashes CAD via the activation of CASP3. This is followed by the translocation of ENDOG and CAD from the cytosol to the nucleus, where these nucleases catalyze DNA cleavage. Upon their generation, spDSBs ignite an ATM/NF-κB/STAT3 cascade that promotes malignant tumorigenicity and stemness.

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References

1 Crasta K, Ganem NJ, Dagher R, *et al*. *Nature* 2012; **482**:53-58.
2 Janssen A, van der Burg M, Szuhai K, *et al*. *Science* 2011; **333**:1895-1898.
3 Zeman MK, Cimprich KA. *Nat Cell Biol* 2014; **16**:2-9.
4 O'Connor MJ. *Mol Cell* 2015; **60**:547-560.
5 Vitale I, Manic G, Senovilla L, *et al*. *Trends Cancer* 2015; **1**:124-135.
6 Marechal A, Zou L. *Cold Spring Harb Perspect Biol* 2013; **5**:a012716.
7 Halazonetis TD, Gorgoulis VG, Bartek J. *Science* 2008; **319**:1352-1355.
8 Maugeri-Sacca M, Bartucci M, De Maria R. *Mol Cancer Ther* 2012; **11**:1627-1636.
9 Kreso A, Dick JE. *Cell Stem Cell* 2014; **14**:275-291.
10 Liu X, Li F, Huang Q, *et al*. *Cell Res* 2017; **27**:764-783.
11 Ichim G, Lopez J, Ahmed SU, *et al*. *Mol Cell* 2015; **57**:860-872.
12 Manic G, Signore M, Sistigu A, *et al*. *Gut* 2017; pii:gutjnl-2016-312623.