EBioMedicine 2 (2015) 1844–1845

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Commentary

Penetrating enemy territory: Soluble PCNA-peptides stress out MYCN-overexpressing neuroblastomas

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Since its first description in 1910 (Wright, 1910), neuroblastoma has emerged as one of the most common cancers in infants less than one year of age. *MYCN* (*MYC* neuroblastoma-derived homolog) amplification was one of the first reliable biomarkers and is to this day an indicator of rapid disease progression and poor prognosis (Huang and Weiss, 2013). High levels of *MYCN* expression strongly correlate with metastatic behavior, and sadly about 20% of high-risk neuroblastoma patients are refractory to chemotherapy. MYCN is a member of the MYC family of transcription factors all of which are homologous to the viral oncogene *v-myc* (for myelocytomatosis). Similar to MYC, MYCN heterodimerizes with MAX at E-box sequences and induces a large number of target genes that drive proliferative responses (Huang and Weiss, 2013). Both MYC and MYCN are difficult to block directly due to their structural specifications, and efforts aimed at functionally inhibiting MYC family members have remained largely unsuccessful.

A study by Gu et al. in this issue of *EBioMedicine* demonstrates that neuroblastomas with high-level *MYCN* amplifications carry a particular burden: they experience chronic replication stress and rely on signaling pathways that counteract the collapse of active replication forks Gu et al. (2015). Replication stress is a collective term for challenges that can arise during S phase and cause replication complexes to arrest. Underlying causes can be the shortage of nucleotides, defects in a component of the replication machinery or insufficient origin activation that results in too few replication forks to complete genome replication (Bielinsky, 2003). All of these deficiencies result in a delay of DNA synthesis that leaves the unwound DNA template single-stranded. The single-stranded DNA is rapidly bound by RPA (replication protein A), which recruits the ATR (ataxia telangiectasia mutated and Rad3-related) kinase in conjunction with its binding partner ATRIP (ATR interacting protein). ATR/ATRIP then promotes the activation of CHK1, the checkpoint kinase that shields arrested forks from collapse and concomitant inactivation (Zeman and Cimprich, 2014).

Gu et al. had previously reported that a soluble peptide, R9-caPep, derived from the interdomain loop of the replication clamp PCNA (proliferating cell nuclear antigen) selectively inhibited the proliferation of neuroblastoma cells that harbored *MYCN* amplifications (Gu et al., 2014). However, the underlying mechanism(s) remained elusive. The present study confirms and significantly extends previous results by the Maris laboratory that demonstrated that neuroblastomas in which *MYCN* was amplified overexpressed and constitutively activated CHK1 (Cole et al., 2011). Gu et al. re-analyzed microarray data from 472 neuroblastoma tumors and examined a total of 36 new patient-derived specimens to validate the notion that high-level *MYCN* expression strongly correlates with chronic replication stress. In a separate set of experiments, the authors show convincingly that depletion of MYCN in neuroblastoma cell lines reduces the basal burden of genome instability several-fold. At the same time, chronic replication stress confers sensitivity to the PCNA-derived peptide R9-caPep. The peptide interferes with normal PCNA function likely by sequestering binding partners whose actions PCNA coordinates during DNA replication (Stoimenov and Helleday, 2009). Importantly, the inhibitory effect of R9-caPep is diminished when *MYCN* expression is downregulated, consistent with the original observation that the peptide is highly effective in neuroblastoma cells in which the *MYCN* locus is amplified.

A key experiment in this new study is a single-molecule fiber analysis that utilizes consecutive labeling of nascent DNA by two different thymidine analogs. When the second label was applied in the presence of the R9-caPep peptide, replication fork progression was significantly reduced. It is highly unlikely that the observed effect is not specific to the peptide sequence, as the authors had previously shown that a scrambled control peptide did not have any inhibitory effect on DNA synthesis nor cell growth (Gu et al., 2014). Importantly, the PCNA-derived peptide synergizes with CHK1 inhibitors to increase genome instability, enhance the activation of so-called backup origins – another hallmark of replication stress – and induce apoptosis in *MYCN* overexpressing neuroblastoma cells. Whether the cells succumb to RPA exhaustion (Toledo et al., 2013), which has been proposed as a molecular mechanism for replication stress-induced cell death, remains to be explored. In the same vein, it remains to be determined whether MYCN drives cells prematurely into S phase, thereby limiting origin licensing and restricting the number of replication forks that can actively contribute to genome duplication. Alternatively, MYC has recently been described to have an active role in replication initiation that is completely independent of its function in transcription (Srinivasan et al., 2013). It is conceivable that MYCN could play a similar role, leading to an overall dysregulation of origin firing that causes replication stress. Both lines of action are not mutually exclusive and could work in concert.

Taken together, the work by Gu et al. suggests that the combination of CHK1 inhibitors and soluble, cell penetrable PCNA-peptides that

DOI of original article: http://dx.doi.org/10.1016/j.ebiom.2015.11.016.
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http://dx.doi.org/10.1016/j.ebiom.2015.12.012
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block replication fork progression could be a promising therapeutic tool to aim at the Achilles heel of *MYCN*-overexpressing neuroblastomas.

Conflict of Interest

The author declares no conflict of interest.

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