EBioMedicine 2 (2015) 621–622

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Commentary

Can we be SMaRT-er in our approach to cancer therapy?

Christina Gruber[1], Josefina Piñón Hofbauer[1], Johann W. Bauer*

Department of Dermatology and EB House Austria, Paracelsus Medical University, Salzburg, Austria

Spliceosome-mediated RNA trans-splicing (SMaRT) is a molecular tool that facilitates genetic reprogramming on the RNA level (Wally et al., 2012). SMaRT exploits the cells own splicing machinery to recombine two RNA molecules: the endogenous RNA target and the RNA trans-splicing molecule (RTM). The end product is a chimeric RNA wherein part of the message encoded by the target RNA is replaced with one provided by the RTM. The specificity of the trans-splicing reaction is conferred by an anti-sense binding domain complementary to a non-coding region within the target RNA. This serves to tether the RTM to its target RNA, bringing donor and acceptor splice sites on both molecules into close proximity thereby allowing the trans-splicing reaction to occur. Since its first demonstration in 1999, where it was used in a cancer suicide gene therapy approach (Puttaraju et al., 1999), SMaRT has primarily made a niche in the field of gene therapy where it has successfully been employed in pre-clinical investigations for the therapy of various genetic disorders, including cystic fibrosis, hemophilia A, spinal muscular atrophy, and the severe skin blistering disease epidermolysis bullosa (as reviewed in Wally et al., 2012).

In this issue, Uckun et al. take the concept of SMaRT-mediated repair back to its roots and explore its use in the repair of an oncogenic defect, CD22ΔE12, in childhood B-precursor leukemia (BPL), the largest subset of B-lineage acute lymphoblastic leukemia (ALL) (Uckun et al., 2015a). BPL cells express dysfunctional CD22, a principal negative regulator of B cell receptor signaling, due to homozygous intronic mutations. These mutations result in aberrant splicing and loss of exon 12, leading to translation pre-termination and the generation of a truncated protein which lacks the regulatory domains required for proper signal transduction and apoptosis induction (Uckun et al., 2010). Forced expression of human CD22ΔE12 in mice resulted in spontaneous development of B-ALL with a gene signature that closely recapitulates that of human ALL, indicating that CD22ΔE12 is an oncogenic driver (Uckun et al., 2015a, 2010). Because CD22ΔE12 is associated with aggressive and chemo-refractory disease, the authors sought to repair this defect by using SMaRT technology to replace the exons 10–14 of the mutant pre-mRNA with the wildtype sequence using a rationally designed RTM (Uckun et al., 2015b). Transfection of the RTM into leukemia-initiating ALL cells significantly reduced their ability to cause leukemia in a xenograft animal model (Uckun et al., 2015a). This effect is presumably due to the repair and restoration of functional CD22, though this remains to be stringently demonstrated. Functional repair of CD22 should be evaluated by investigating cellular outcomes in response to appropriate B cell signals. Additionally, a reversion of the gene expression signature in RTM-treated cells towards that of wild type would further confirm functional CD22 repair as the mechanism underlying the anti-cancer effects observed.

These studies follow at the heels of He et al., who used the same SMaRT technology to repair mutant p53 transcripts in hepatocellular carcinoma leading to restored wild type p53 function and suppression of tumor growth in vivo (He et al., 2015). Together, both these studies pose several interesting questions. Is it possible to treat cancer by repairing the lesions that drive it? What efficiencies need to be achieved – at the level of RTM delivery to cancer cells, and at the level of repair – in order to achieve an anti-cancer effect?

While for most gene therapy applications, a 100% repair is likely not necessary (Chao et al., 2003), one would expect that in the context of cancer therapy, a higher efficiency is essential to prevent clonal expansion of single tumor cells that escape treatment. This may apply particularly to SMaRT when used in the context of a suicide gene therapy approach (Gruber et al., 2013). One way to boost trans-splicing over the competing cis-splicing reaction is via the use of antisense oligonucleotides that mask cis-splicing promoting elements (Koller et al., 2015). The repair of the faulty mRNA is expected to reduce the overall expression of the mutated protein while increasing the levels of wild type protein. This shift may be enough to reduce chemoresistance, rendering even partially-corrected cells susceptible to conventional treatments, so that combination regimens that include SMaRT should be considered for future investigations. It is important that future efforts also focus on specificity issues including possible off target effects such as unspecific trans-splicing, and cryptic cis-splicing or undesirable translation from within the RTM (Gruber et al., 2013; Monjaret et al., 2014). The use of next generation RNA sequencing technologies should aid in the in-depth evaluation of SMaRT specificity. Finally, the bottleneck for any gene therapy approach is the in vivo delivery of the therapeutic DNA or RNA. In this respect, we have seen an increasing number of reports on the use of non-viral polymer-based nanoparticles that protect therapeutic DNA molecules from degradation and deliver them into the target cells with high efficiency, such as those reported by Uckun et al. When fully evaluated for safety and efficacy, these will likely be preferred over viral delivery methods. However, further efforts should focus on specifically targeting these particles to cancer cells, especially for cancer types were systemic application is necessary. Moreover,

DOI of original article: http://dx.doi.org/10.1016/j.ebiom.2015.04.016.

* Corresponding author at: Department of Dermatology and EB House Austria, Paracelsus Medical University, Muellner Hauptstrasse 48, 5020 Salzburg, Austria.

E-mail address: jo.bauer@salk.at (J.W. Bauer).

[1] Equal contributors.

http://dx.doi.org/10.1016/j.ebiom.2015.06.002

rigorous testing of the effects of the RTM in would-be bystander cells is highly recommended.

Advances in the next generation sequencing platforms, and RNAi screens, continue to spearhead the identification and validation of lesions that drive tumorigenesis and confer chemoresistance to malignant cells. In parallel, today's arsenal of genetic editing technologies, including SMaRT, TALENs, and CRISPR, are continuously improving. While these technologies are under intense development for the repair of mutations in monogenic disorders, they are hardly explored as anti-cancer strategies. It is perhaps precisely because cancer is not a monogenic disease, that we have not considered the concept of repairing a single dominant lesion as a potent anti-cancer strategy. Yet, if the results of these recent studies stand up to critical cross-examination, it could be a concept worth further exploration, potentially extending the array of personalized cancer medicines.

Conflict of interest

JWB is co-inventor on US 8,735,366 B2: Pre-MRNA trans-splicing molecule (RTM) molecules and their uses. Otherwise the authors state no conflict of interest.

Acknowledgments

CG and JPH are supported by DEBRA Austria.

References

Chao, H., Mansfield, S.G., Bartel, R.C., et al., 2003. Phenotype correction of hemophilia A mice by spliceosome-mediated RNA trans-splicing. Nat. Med. 9, 1015–1019.
Gruber, C., Koller, U., Murauer, E.M., et al., 2013. The design and optimization of RNA trans-splicing molecules for skin cancer therapy. Mol. Oncol. 7, 1056–1068.
He, X., Liu, F., Yan, J., et al., 2015. Trans-splicing repair of mutant p53 suppresses the growth of hepatocellular carcinoma cells in vitro and in vivo. Sci. Rep. 5, 8705.
Koller, U., Hainzl, S., Kocher, T., et al., 2015. Trans-splicing improvement by the combined application of antisense strategies. Int. J. Mol. Sci. 16, 1179–1191.
Monjaret, F., Bourg, N., Suel, L., et al., 2014. Cis-splicing and translation of the pre-trans-splicing molecule combine with efficiency in spliceosome-mediated RNA trans-splicing. Mol. Ther. 22, 1176–1187.
Puttaraju, M., Jamison, S.F., Mansfield, S.G., et al., 1999. Spliceosome-mediated RNA trans-splicing as a tool for gene therapy. Nat. Biotechnol. 17, 246–252.
Uckun, F.M., Goodman, P., Ma, H., et al., 2010. CD22 EXON 12 deletion as a pathogenic mechanism of human B-precursor leukemia. Proc. Natl. Acad. Sci. U. S. A. 107, 6852–6857. http://dx.doi.org/10.1073/pnas.1007896107.
Uckun, F.M., Mitchell, L.G., Qazi, S., et al., 2015a. Development of polypeptide-based nanoparticles for non-viral delivery of CD22 RNA Trans-splicing molecule as a new precision medicine candidate against B-lineage ALL. EBioMedicine 2, 647–657.
Uckun, F.M., Qazi, S., Ma, H., et al., 2015b. CD22DeltaE12 as a molecular target for corrective repair using RNA trans-splicing: anti-leukemic activity of a rationally designed RNA trans-splicing molecule. Integr. Biol. (Camb.) 7, 237–249.
Wally, V., Murauer, E.M., Bauer, J.W., 2012. Spliceosome-mediated trans-splicing: the therapeutic cut and paste. J. Invest. Dermatol. 132, 1959–1966.