Letter to the Editor

Characterization of extrachromosomal circular DNA in patients with acute myeloid leukemia: proof-of-concept report using cohorts from Beijing and Shanghai

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Comment on: Sun Z, Ji N, Zhao R, *et al.* Extrachromosomal circular DNAs are common and functional in esophageal squamous cell carcinoma. Ann Transl Med 2021;9:1464.

Submitted Mar 20, 2022. Accepted for publication Jul 08, 2022.
doi: 10.21037/atm-22-1498
View this article at: https://dx.doi.org/10.21037/atm-22-1498

Circular DNA is a form of DNA molecules commonly found in nature, like genomic DNA of microorganism, bacterial plasmids, or mitochondrial DNA. As these are DNA molecules that exist independently outside the chromosomes and circular structures, they are called extrachromosomal circular DNA (eccDNA) (1).

We have read with great interest the manuscript of Sun *et al.* (2), in which they proved the gone-wide presence of extrachromosomal circulating DNA and showed its potential in the pathogenesis of esophageal squamous cell carcinoma. EccDNA elements are responsible for carrying DNA sequences that are homologous with demonic DNA (3). Still, they are different from mitochondrial DNA (4), as well as different from circular DNA that is viral covalently closed (5). Various reports investigated the link between eccDNA and cancer biology, as eccDNA is a potential biomarker for cancer monitoring and therapy.

Circular DNA is commonly chimeric circularized and amplified, thus greatly impacting the enhanced expression of oncogenes. Still, published data has yet to clarify whether the circularization *per se* or the subsequent amplification of the copy number may lead to the upregulation of various oncogenes. So far, progress in genetics showed additional roles of eccDNA, other than to promote the amplification and transcription of oncogenes. It may lead to oncogenic remodeling in human malignancies, including leukemia, with important clinical impact (6).

As there are still several unanswered questions regarding AML biology, eccDNAs can offer a better understanding of this. Using Chinese cohorts from Shanghai and Beijing, in a collaborative experiment between China and Romania, in the current study we observed 298 upregulated eccDNAs and 71 downregulated eccDNAs in AML patients compared to healthy controls. When considering only eccDNAs from known genes, we observed 273 upregulated eccDNAs and 37 downregulated eccDNAs, most of which were from protein-coding genes (*Figure 1*). We found a cluster of genes with the P value of 0 (considered by the software) and with a logFC of 1.74. All these eccDNAs were derived from the mitochondrial genome. It must be mentioned that

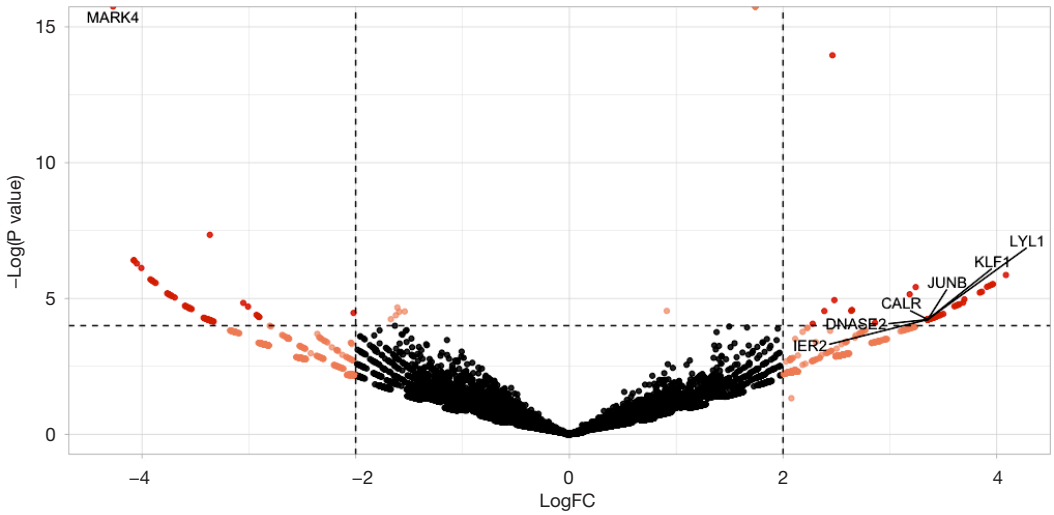

Figure 1 Volcano plot of the assessed eccDNAs. eccDNA, extrachromosomal circular DNA.

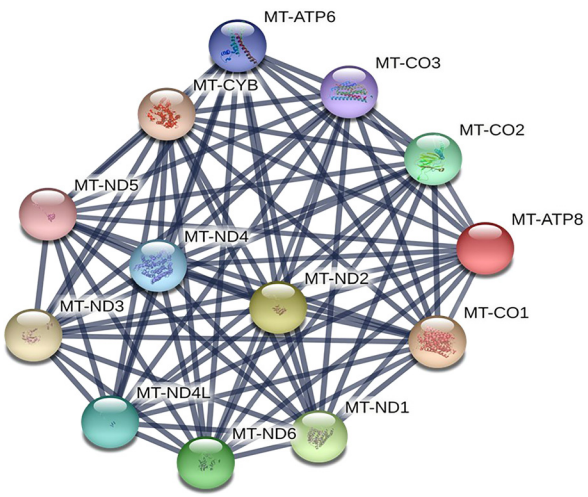

Figure 2 Mitochondrial eccDNAs. eccDNA, extrachromosomal circular DNA.

these were all the mitochondrial genome-derived eccDNAs detected (*Figure 2*).

When assessing the upregulated protein-coding eccDNAs, we prove an enrichment in genes involved in myeloid cell differentiation (*JUNB*, *DNASE2*, *KLF1*), as well as genes involved in cell differentiation (*JUNB*, *DNASE2*, *KLF1*, *CALR*, *IER2*, *LYL1*) (*Figure 3*). When assessing the upregulated processes using STRING, several genes implicated in DNA binding stand out. Conversely, when assessing the downregulated eccDNAs, we did not observe any processes when using GOrilla, nor any discernable network when using STRING (*Figure 4*).

In the current study, we present the differences of the eccDNA content between AML patients and healthy controls. We show that the percentage of the non-protein coding eccDNAs from the total eccDNAs is higher in downregulated eccDNAs, when compared to the upregulated ones. We also report that all eccDNAs derived from the mitochondrial genome were upregulated in AML patients. This might potentially prove the difference in mitochondrial activity between AML patients and healthy controls. This is in accordance with the literature as it shows that there are alterations in the mitochondrial processes in malignancies in general and in AML specifically.

Moreover, of all the upregulated eccDNAs derived from genes involved in myeloid differentiation, we must mention JunB proto-oncogene (JUNB), as this gene has been recurrently shown to be implicated in the biology of AML. Thus, the eccDNA derived from JUNB might influence the activity of this gene and, thus, an indirect influence in the biology of AML (7-9).

Of note, we must mention that the downregulated eccDNAs were derived from genes which did not form a network, and this shows that there might be far more importance in AML regarding the upregulation of eccDNA.

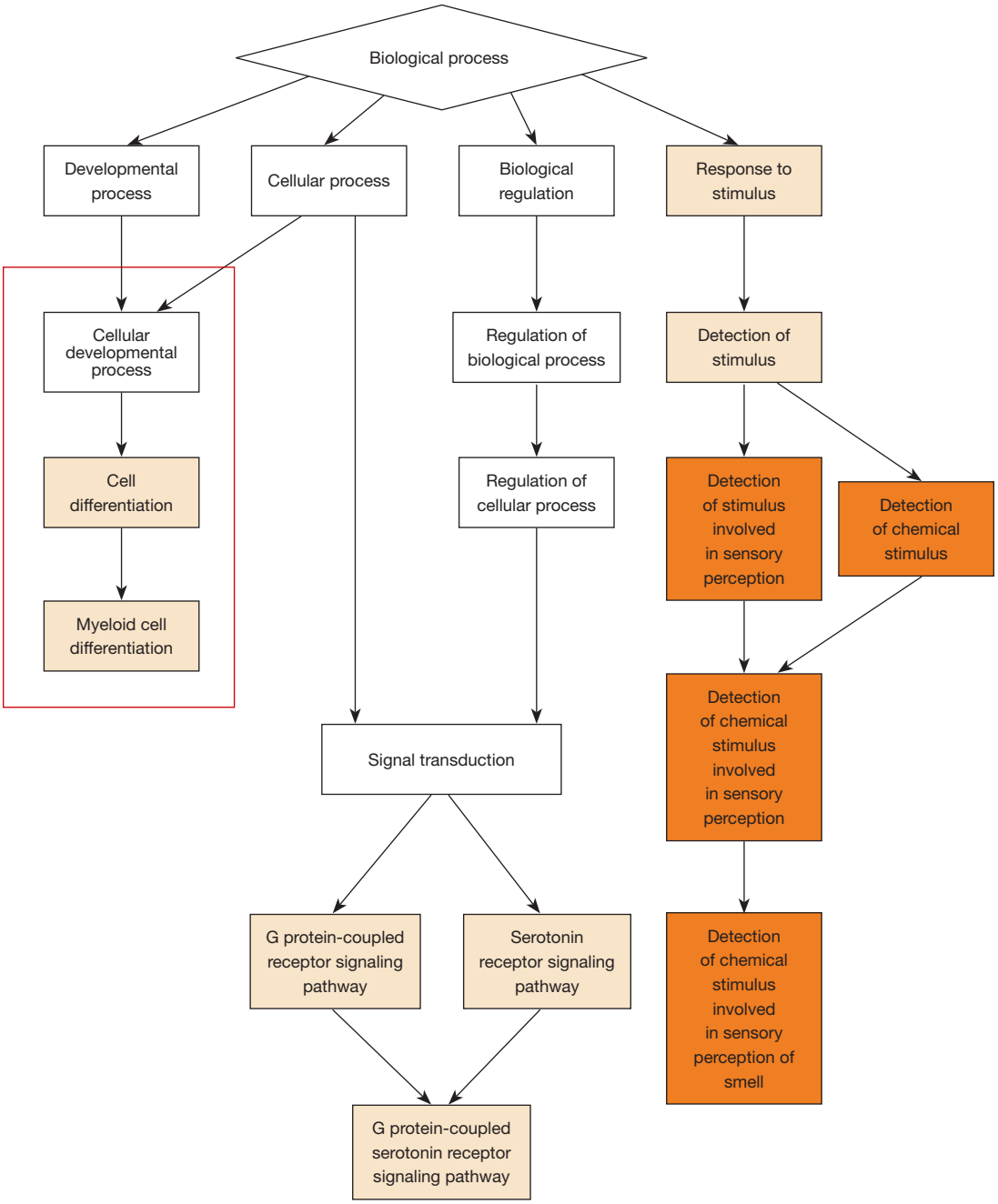

Figure 3 Upregulated processes using the GOrilla software.

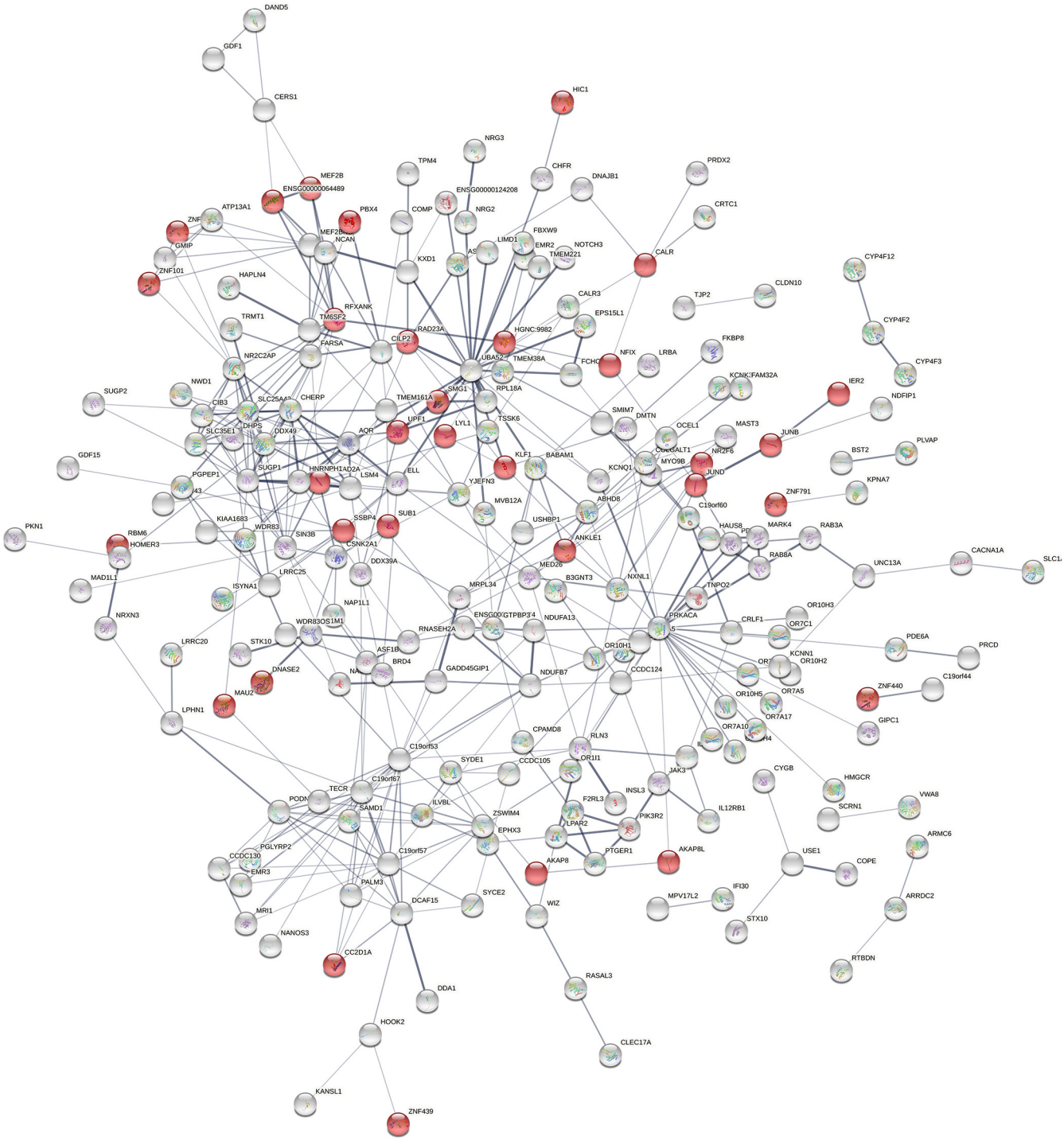

Figure 4 Upregulated processes using the STRING software. Genes involved in DNA binding are marked with red.

Acknowledgments

Funding: This work was supported by a grant from the National Defense Science and Technology Innovation Project (Grant No. 20-163-00-TS-009-006-01), the National Natural Science Foundation of China (Grant No. 81800180), and the National Young Elite Scientists Sponsorship Program by China Association for Science and Technology (Grant No. 17-JCJQ-QT-032); as well as by grants from the Romanian Ministry of Research and Innovation: CCCDI-UEFISCDI, Project No. PN-III-P4-ID-PCCF-2016-0112 within PNCDI III, for Young Research Teams 2020-2022 (Grant No. PN-III-P1-1.1-TE-2019-0271); PN-III-P4-ID-PCE-2020-1118 within PNCDI IV, Projects for Exploratory Medicine; PN-III-CEI-BIM-PBE-2020-0016 within PNCDI I – collaboration between Romania and Belgium (Wallonia), contract number 13-BM/2020; Project of Postdoctoral Research PN-III-P1-1.1-PD-2019-805, contract No. PD122/18.08.2020; Project Experimental-Demonstrativ PN-III-P2-2.1-PED-2019-3640, contract No. 539PED/26.10.2020; CNCS-UEFISCDI, project PN-III-P1-1.1-TE-2016-0919; as well as by an international collaborative grant of the European Economic Space between Romania and Iceland 2021-2023 "Cooperation strategy for knowledge transfer, internationalization and curricula innovation in the field of research education at the 3rd level of study—AURORA".

Footnote

Provenance and Peer Review: This article was a standard submission to the journal. The article did not undergo external peer review.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-1498/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Paulsen T, Kumar P, Koseoglu MM, et al. Discoveries of Extrachromosomal Circles of DNA in Normal and Tumor Cells. Trends Genet 2018;34:270-8.
2. Sun Z, Ji N, Zhao R, et al. Extrachromosomal circular DNAs are common and functional in esophageal squamous cell carcinoma. Ann Transl Med 2021;9:1464.
3. Møller HD, Ramos-Madrigal J, Prada-Luengo I, et al. Near-Random Distribution of Chromosome-Derived Circular DNA in the Condensed Genome of Pigeons and the Larger, More Repeat-Rich Human Genome. Genome Biol Evol 2020;12:3762-77.
4. Dennin RH. Overlooked: Extrachromosomal DNA and Their Possible Impact on Whole Genome Sequencing. Malays J Med Sci 2018;25:20-6.
5. Huang JT, Yang Y, Hu YM, et al. A Highly Sensitive and Robust Method for Hepatitis B Virus Covalently Closed Circular DNA Detection in Single Cells and Serum. J Mol Diagn 2018;20:334-43.
6. Ott CJ. Circles with a Point: New Insights into Oncogenic Extrachromosomal DNA. Cancer Cell 2020;37:145-6.
7. Basak NP, Banerjee S. Mitochondrial dependency in progression of acute myeloid leukemia. Mitochondrion 2015;21:41-8.
8. Zhou C, Martinez E, Di Marcantonio D, et al. JUN is a key transcriptional regulator of the unfolded protein response in acute myeloid leukemia. Leukemia 2017;31:1196-205.
9. Somervaille TC, Cleary ML. PU.1 and Junb: suppressing the formation of acute myeloid leukemia stem cells. Cancer Cell 2006;10:456-7.

Cite this article as: Qian L, Xia X, Liu J, Chen X, Liu Y, Wang X, Iluta S, Pasca S, Gulei D, Tomuleasa C. Characterization of extrachromosomal circular DNA in patients with acute myeloid leukemia: proof-of-concept report using cohorts from Beijing and Shanghai. Ann Transl Med 2022;10(15):843. doi: 10.21037/atm-22-1498

