Genes & Diseases (2024) 11, 532–534

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.keaipublishing.com/en/journals/genes-diseases

RAPID COMMUNICATION

Identification and global characterization of eccDNA reveals hallmarks in iron nanoparticles-treated breast cancer cells

The biogenesis and functions of extrachromosomal circular DNA (eccDNA) have been studied for several decades. However, the heterogeneity of eccDNA is largely ignored. In this study, we purified and sequenced eccDNA and RNA using a method that simultaneously extracts DNA and RNA from cultured cells treated with iron nanoparticles. We discovered that the characteristics of eccDNA are altered by iron stress, particularly on genic and CpG islands regions, suggesting a biogenesis mechanism induced by transcription. However, we found that only a few eccDNA-annotated genes were shared by differentially expressed genes identified by RNA-seq, indicating that eccDNA has a complex background that may be the result accumulated from previous states. In addition, we observed little variation in the distribution pattern of eccDNA in ferroptosis cells with fewer differentially expressed genes, indicating that eccDNA is generated randomly in cells when they are unable to regulate many more genes to resist environmental stress. Collectively, our study characterized eccDNA induced by iron stress, uncovered their intricate background, and proposed the alterations in the distribution pattern of eccDNA as a potential biomarker.

EccDNA is circular DNA and is found in both normal and tumor cells.[1] Substantial evidence suggests that the biogenesis and functions of eccDNA are associated with transcription aimed at adaptation.[2] However, this view is controversial.[3] Moreover, several studies have demonstrated that among the hundreds of thousands of eccDNAs in each sample replicate, only a few to a dozen eccDNAs share identical junction sites or overlapping sequences.[4] Therefore, we suspect that DNA and RNA samples generated using distinct protocols ignore the heterogeneity of eccDNA in previous research. Consequently, transcriptome data may not correspond to eccDNA data obtained from its replicates. To investigate the eccDNA profiles and explore the association between transcriptome and eccDNA under various conditions, we induced iron metabolism in cells using iron nanoparticles to assess the hypothetical transcriptional activity. Furthermore, under the same concentration of iron nanoparticles, the gene interfered-ferroptosis therapy (GIFT) method[5] was used to induce ferroptosis through inhibition of the genes exporting intracellular iron ions to test the hypothesis that the origin of eccDNA is related to cell death. Simultaneously, we utilized the co-extraction method (Fig. 1A) to isolate eccDNAs from MCF-12 A and MDA-MB-453 cells treated with or without DMSA-coated Fe_3O_4 nanoparticles (FeNPs).

Circle size inspection revealed that the distribution of eccDNAs was concentrated between 0.1 kb and 10 kb, with ~160–200 bp regular intervals (Fig. 1B; Fig. S1), indicating that eccDNAs may be derived from open chromatin regions where gene transcription occurs. Although eccDNAs were detected on all chromosomes, we found a significant increase on chromosomes 16, 17, 19, and 20 in FeNPs-treated cells (Fig. 1C). We further examined the normalized counts of eccDNAs and the number of coding genes on each chromosome (Fig. 1D). Intriguingly, although there was no significant correlation in our study, we found an increased positive correlation tendency in iron-stressed cells, suggesting that the correlation between the number of eccDNAs and the number of coding genes was variable and that more eccDNAs were generated from coding gene regions along with transcriptional activation in response to different environmental stress. We annotated the eccDNA molecules under iron stress were significantly increased in 5′-untranslated regions (5′-UTRs), 3′-untranslated regions (3′-UTRs), exonic regions, and CpG island regions, which were associated with transcription and promoters (Fig. 1E). On the other hand, there was no significant difference in the genomic distributions of eccDNAs in intron and intergenic regions whether iron nanoparticles were present or

Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2023.04.002

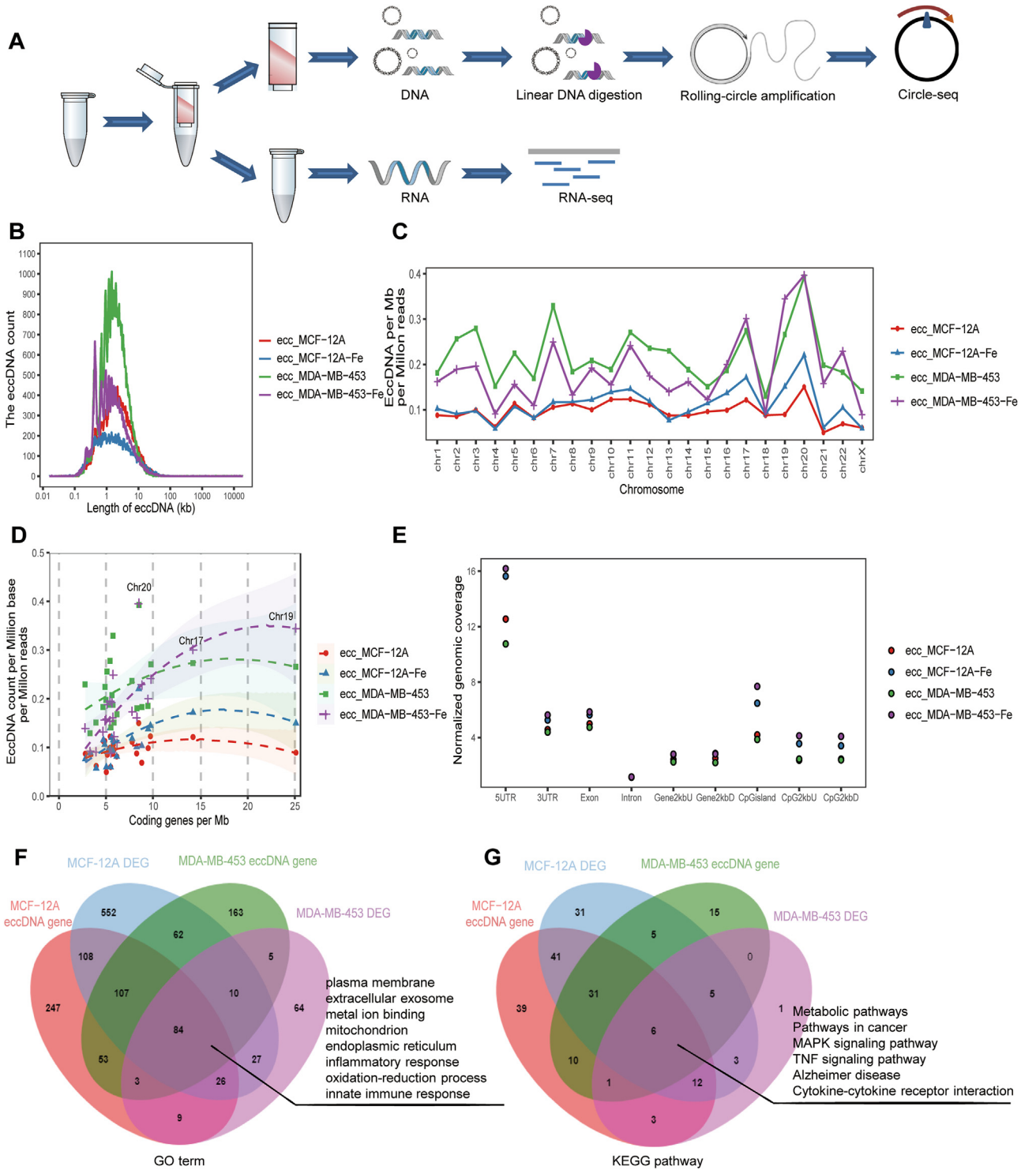

Figure 1 Characterization of eccDNAs in FeNPs-treated cells. **(A)** Schematic illustration of the eccDNA and RNA co-extraction and sequencing procedure. **(B)** Length distribution of eccDNAs. **(C)** Normalized counts of eccDNAs on each chromosome. **(D)** The relationship between normalized counts of eccDNAs and coding genes per Mb on each chromosome. **(E)** The distribution of eccDNAs in different genomic elements. Gene2kbU, 2 kb upstream of genes; Gene2kbD, 2 kb downstream of genes; CpG2kbU, 2 kb upstream of CpG islands; CpG2kbD, 2 kb downstream of CpG islands. **(F, G)** Four-way Venn analysis of GO terms and KEGG pathways in the four groups.

not. Such variable patterns showed a correlation between transcription and promoters and eccDNA generation.

By comparing FeNPs-treated and untreated cells for differentially expressed protein-coding genes (DEGs) and eccDNA-annotated genes, we discovered that a few eccDNA-annotated genes overlapped with DEGs (Fig. S2). This finding suggested that eccDNAs cannot always be produced from significantly differentially expressed gene regions. Further, we evaluated all the GO terms and KEGG pathways for eccDNA-annotated genes and DEGs (Fig. 1F, G). We demonstrated that approximately half of the GO terms and KEGG pathways were shared, indicating that the eccDNAs produced in FeNPs-treated cells were related to several biological processes that occur in iron-stressed cells.

However, the chromosome distribution of eccDNAs in GIFT-treated MDA-MB-453 cells was comparable to that of non-treated cells, even on chromosomes 16, 17, 19, and 20 (Fig. S3A). Moreover, the genomic element distributions of eccDNAs were comparable to those of untreated cells (Fig. S3B). Other than a considerable increase in 5′UTR, the distributions of eccDNAs did not differ significantly in other elements such as 3′UTR. In comparison to eccDNAs detected in FeNPs-treated cells, eccDNAs identified in GIFT-treated cells were significantly enhanced on each chromosome and exhibited a comparable distribution pattern on chromosomes and genomic elements. Eventually, only 147 differentially DEGs were identified in the GIFT-treated group (Fig. S3C). Comparatively, the chromosome distribution and the genomic element distributions of eccDNAs in erastin-treated MCF-12 A exhibited a similar trend as those in FeNPs-treated cells (Fig. S3D, E). In addition, a total of 2068 differentially expressed encoding genes were screened in erastin-treated MCF-12 A (Fig. S3F), which was far more than GIFT-treated MDA-MB-453 and had a considerable amount of DEGs compared with FeNPs-treated cells. We hypothesized that the characteristics of the eccDNAs identified in ferroptotic MDA-MB-453 cells were remarkably like those of previously treated cells because there were not a large number of eccDNAs produced by specific transcription as the cells were unable to regulate many more genes to resist ferroptosis, and exhibited a near-random distribution of eccDNA throughout the entire genome (Fig. S4).

This study characterized eccDNAs and investigated the association between eccDNA and transcription. Additionally, we observed a pattern of regularity in the global distribution of eccDNA changes, suggesting that eccDNA could serve as a biomarker.

Conflict of interests

The authors have declared no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.04.002.

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(4):270–278.
2. Zuo S, Yi Y, Wang C, et al. Extrachromosomal circular DNA (eccDNA): from chaos to function. *Front Cell Dev Biol*. 2022;9:792555.
3. Møller HD, Mohiyuddin M, Prada-Luengo I, et al. Circular DNA elements of chromosomal origin are common in healthy human somatic tissue. *Nat Commun*. 2018;9(1):1069.
4. Zhu J, Zhang F, Du M, et al. Molecular characterization of cell-free eccDNAs in human plasma. *Sci Rep*. 2017;7(1):10968.
5. Gao J, Luo T, Wang J. Gene interfered-ferroptosis therapy for cancers. *Nat Commun*. 2021;12(1):5311.

Wenxiang Lu [a], Ying Wang [a], Tao Luo [a], Fuyu Li [a], Yali Jiang [b], Jinke Wang [a], Weizhong Zhang [b,c,**], Yunfei Bai [a,*]

[a] *State Key Lab of Bioelectronics, School of Biological Science and Medical Engineering, Southeast University, Nanjing, Jiangsu 210096, China*

[b] *The Friendship Hospital of Ili Kazakh Autonomous Prefecture, Ili & Jiangsu Joint Institute of Health, Yining, Xinjiang 835000, China*

[c] *Department of Ophthalmology, First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu 210029, China*

*Corresponding author.

**Corresponding author. The Friendship Hospital of Ili Kazakh Autonomous Prefecture, Ili & Jiangsu Joint Institute of Health, Yining, Xinjiang 835000, China.

E-mail addresses: weizhongzhang@njmu.edu.cn (W. Zhang), whitecf@seu.edu.cn (Y. Bai)

9 December 2022
Available online 8 May 2023