

RESEARCH LETTER

The Significance of Polyploid Hepatocytes During Aging Process



The mammalian liver contains numerous polyploid hepatocytes, and polyploidization is significantly enhanced during aging.¹ In some tissues, such as bone marrow, diploid stem cells constitutively proliferate for tissue renewal and regeneration, whereas polyploidization leads to cell cycle arrest and differentiation enhancing cellular functions.² In contrast, notably, polyploid hepatocytes are an important source of liver regeneration under the stress conditions.³ Polyploidy in hepatocytes has also been hypothesized to contribute to enhancing metabolic functions, and could influence functional properties of the liver.⁴ However, it has been unknown how polyploid hepatocytes play roles in physiological cellular renewal and impact on liver (dys)functions during the aging process.

To examine whether polyploid hepatocytes contribute to cellular renewal during normal aging, livers of aged Rosa-*RGBow*^{+/-} mice⁵ (27 months old) were compared with their young 3-month-old counterparts (Figure 1A). The Rosa-*RGBow* allele consists of multiple fluorescent reporter genes, and a subset of polyploid cells are labeled as bicolored cells after Cre recombination in heterozygous Rosa-*RGBow* mice (Figure 1A).³ As we previously showed,³ rAAV8-Ttr-Cre (6×10^{10} vector genomes) efficiently (about 75%) and specifically labeled hepatocytes in adult Rosa-*RGBow*^{+/-} mice (Figure 1B, Supplementary Figure 1A), and all bicolored hepatocytes were polyploid (Supplementary Figure 1B). Interestingly, aged livers at >700 days after rAAV8-Ttr-Cre administration contained a substantial number of bicolored hepatocytes at a frequency similar to young livers (Figure 1C). Because murine hepatocytes divide every 200–300 days during normal homeostasis, this result suggests that abundant polyploid

hepatocytes, which account for >90% of hepatocytes in the liver, repeatedly divide to maintain normal turnover of hepatocytes during aging. Indeed, young hepatocytes underwent further polyploidization during aging by going through additional cell cycles (Figure 1D).

Furthermore, microscopic analysis revealed that both young and aged livers exhibited a similar bias of distribution of bicolored hepatocytes among liver zones. More bicolored hepatocytes were consistently located in pericentral zone 3 than in periportal zone 1 in both young and aged mice (Figure 1B and E). This is consistent with a biased distribution of diploid hepatocytes to the periportal area,⁶ and argues against dynamic migration of hepatocytes from zone 1 to zone 3 or vice versa during aging. Importantly, despite the constant frequencies of bicolored hepatocytes during aging, binucleated cells among bicolored hepatocytes were significantly fewer in the aged liver than in the young

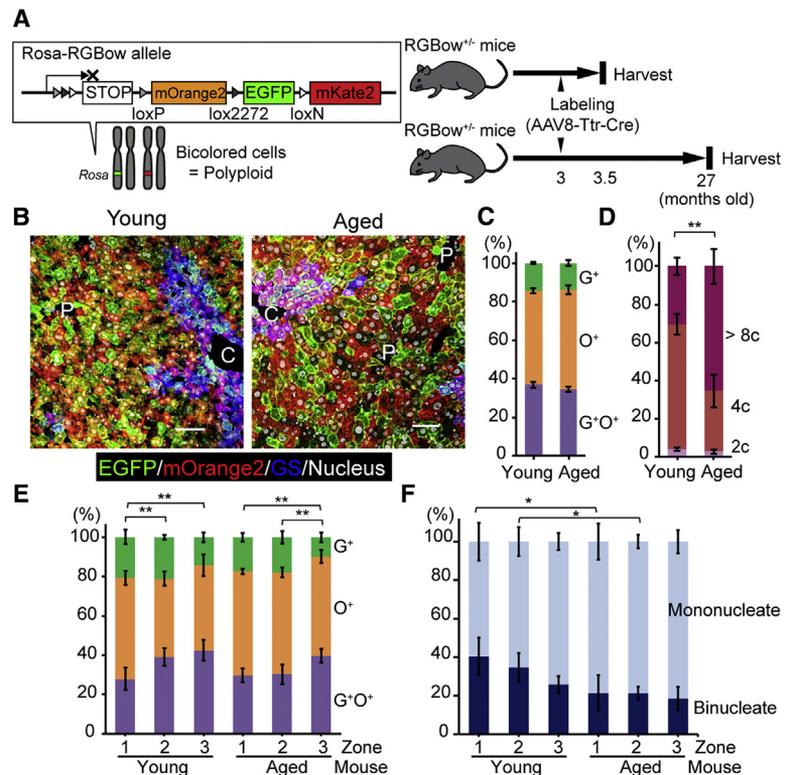


Figure 1. Polyploid hepatocytes serve as the source of cellular turnover during aging. (A) Experimental overview. (B) Microscopic images of young and aged Rosa-*RGBow*^{+/-} mouse livers. Rosa-*RGBow* reporters are expressed in a membrane-bound manner, and mKate2 is not shown because its expression is quite rare (<2% of labeled cells). Single color images are shown in Supplementary Figure 1A. Scale bars, 100 μ m. C, central veins; P, portal veins. (C) Frequencies of labeled hepatocytes. (D) Ploidy distribution of hepatocytes. C and D were analyzed by flow cytometry. (E) Frequencies of labeled hepatocytes in each liver zone. (F) Frequencies of mononucleated and binucleated cells among bicolored hepatocytes. E and F were analyzed by microscopy. Error bars indicate standard deviations (n = 4). **P* < .05, ***P* < .01. G+, EGFP+; O+, mOrange+.

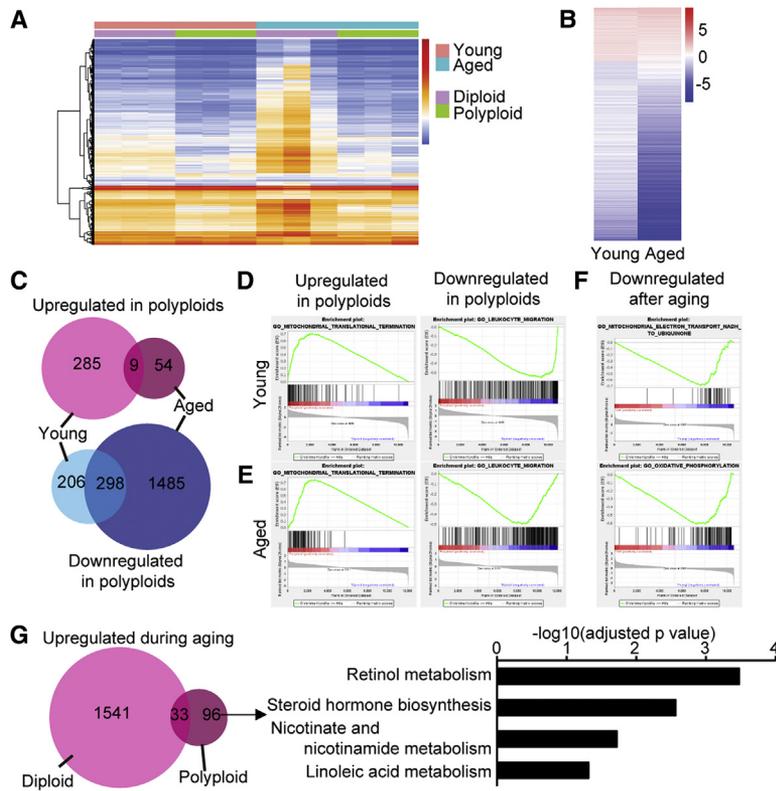


Figure 2. Impacts of aging on gene expressions of polyloid hepatocytes. (A) Heatmap of RNA sequencing data. (B) Heatmap of differentially expressed genes between diploid and/or polyloid hepatocytes. (C) Venn diagrams of differentially expressed genes that were upregulated or downregulated in polyloid hepatocytes compared with diploid counterparts. (D, E) Examples of gene sets enriched in polyloid and diploid hepatocytes. (D and E are derived from young and aged liver, respectively). (F) Examples of gene sets enriched in aged polyloid hepatocytes compared with young counterparts. (G) Pathways upregulated in 96 differentially expressed genes that were uniquely upregulated in polyloids after aging.

liver (Figure 1F). Given that hepatocyte polyploidization occurs via cytokinesis failure and generates binucleated cells at first,⁷ these findings indicate that bicolored binucleated polyploidized cells underwent mitoses with complete cytokinesis, and generated mononucleated cells over time. Taken together, these findings suggest that polyploid hepatocytes proliferate and are an important contributor to maintenance of the liver during normal aging.

Next, the impact of polyploidization on aging-related functional changes of hepatocytes was analyzed by RNA sequencing using diploid and polyloid hepatocytes sorted from young and aged livers (Supplementary Figure 2C and D). Heatmap and principal component analysis showed that each sample exhibited similar gene expression patterns among the 4 groups analyzed (Figure 2A, Supplementary Figure 1E).

The global pattern of gene expression changes between diploid and polyloid hepatocytes was similar in young and aged livers (Figure 2B). Defining differentially expressed genes as those with $P < .05$ and absolute value of \log_2 fold

change >1 identified hundreds of common differentially expressed genes especially in downregulated genes in polyloids (Figure 2C, Supplementary Table 1). Gene set enrichment analysis revealed that genes related to immune responses were commonly downregulated in polyloids compared with diploids both in young and aged livers, whereas genes associated with mitochondrial functions were commonly upregulated in polyloids (Figure 2D and E, Supplementary Table 2). Interestingly, downregulation of immune-related genes and upregulation of genes activating mitochondrial functions were previously reported in decidual polyloids in the uterus,⁸ suggesting that polyloid cells exhibit somewhat similar expressional characteristics regardless of cell types and animal age.

Influence of aging on polyloid hepatocytes was further examined by comparing bicolored polyloid hepatocytes in aged livers with polyloid counterparts in young livers. Because aged livers were labeled about 2 years before harvest, bicolored cells

in aged livers had been polyloid for a long time. Interestingly, gene set enrichment analysis found that mitochondrial function genes were significantly downregulated in polyloids after aging, whereas there was no significant gene ontology upregulated in aged polyloids (Figure 2F, Supplementary Table 3). Mitochondrial dysfunction is one of the features of aging,⁹ and given that mitochondrial function genes are upregulated in polyloids at baseline, polyloid cells may be predisposed to mitochondrial dysfunction during aging. However, 96 differentially expressed genes that were uniquely upregulated in polyloids after aging were significantly enriched with genes annotated to lipid and fatty acid metabolism by pathway analysis, which was consistent with a previous report that compared unsorted young and aged liver tissues (Figure 2G, Supplementary Table 3).¹⁰ Aging-related metabolic changes of the liver may be mainly attributed to that of polyloid hepatocytes.

In summary, we showed here that the abundantly present polyloid

hepatocytes in the mouse liver are the source of physiological hepatocyte turnover during aging. No evidence for a dominant contribution of a diploid liver stem cell during aging was found. Similar processes may occur in the polyploids of other organs with slow cellular turnover, such as acinar cells in the pancreas. In addition, polyploids exhibited characteristic gene expressions compared with diploids, which may be predetermined irrespective of cell types. Moreover, polyploidy-related upregulation of mitochondrial function genes was especially impaired during aging, and polyploid hepatocytes showed higher expressions of lipid and fatty acid metabolic genes than young cells. Although the mechanisms that link polyploidy and age-related liver dysfunction need further investigation, findings here underscore the importance of polyploid hepatocytes in aging-related metabolic liver diseases.

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Conflicts of interest

This author discloses the following: Oregon Health and Science University and Markus Grompe have a significant financial interest in Yecuris, Corp, Tigard, Oregon a company that may have a commercial interest in the results of this research and technology. This potential conflict of interest has been reviewed and managed by Oregon Health and Science University. The other authors disclose no conflicts.

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