



Article

Redox-Dependent Modulation of Human Liver Progenitor Cell Line Fate

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Abstract: Redox homeostasis is determinant in the modulation of quiescence/self-renewal/differentiation of stem cell lines. The aim of this study consisted of defining the impact of redox modifications on cell fate in a human hepatic progenitor line. To achieve this, the HepaRG cell line, which shows oval ductular bipotent characteristics, was used. The impact of redox status on the balance between self-renewal and differentiation of HepaRG cells was investigated using different methodological approaches. A bioinformatic analysis initially proved that the trans-differentiation of HepaRG toward bipotent progenitors is associated with changes in redox metabolism. We then exposed confluent HepaRG (intermediate differentiation phase) to oxidized (H₂O₂) or reduced (N-acetylcysteine) extracellular environments, observing that oxidation promotes the acquisition of a mature HepaRG phenotype, while a reduced culture medium stimulates de-differentiation. These results were finally confirmed through pharmacological modulation of the nuclear factor (erythroid-derived 2)-like 2 (NRF2), a principal modulator of the antioxidant response, in confluent HepaRG. NRF2 inhibition led to intracellular pro-oxidative status and HepaRG differentiation, while its activation was associated with low levels of reactive species and de-differentiation. In conclusion, this study shows that both intra- and extracellular redox balance are crucial in the determination of HepaRG fate. The impact of redox status in the differentiation potential of HepaRG cells is significant on the utilization of this cell line in pre-clinical studies.

Keywords: redox balance; HepaRG; liver regeneration

Citation: Bellanti, F.; Mangieri, D.; di Bello, G.; Lo Buglio, A.; Pannone, G.; Pedicillo, M.C.; Fersini, A.; Dobrakowski, M.; Kasperczyk, A.; Kasperczyk, S.; et al. Redox-Dependent Modulation of Human Liver Progenitor Cell Line Fate. *Int. J. Mol. Sci.* **2023**, *24*, 1904. <https://doi.org/10.3390/ijms24031904>

Academic Editor: Peter Schlemmer

Received: 8 January 2023

Revised: 13 January 2023

Accepted: 16 January 2023

Published: 18 January 2023



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1. Introduction

Stem cells rely on their capacity to maintain pluripotency, self-renew, or activate/differentiate to promote regeneration on intricate signals in their niches. Characterized by special regenerative properties, the liver is able to respond to different stimuli, activating several pathways for mass restoration; indeed, acute organ loss induces hepatocellular proliferation characterized by phenotypic fidelity (canonical pathway), while chronic hepatic injury may activate the trans-differentiation of parenchymal cells or activation of facultative progenitors (alternative pathways) [1,2]. In particular, this latter regenerative mechanism involves a bipotent cell line located in the smallest branches of the ductular tree (regenerative niche), capable of differentiating toward hepatocyte or biliary cell lineages [3–6].

To study regeneration and differentiation, the HepaRG cell line was used as an alternative to progenitors showing human oval ductular bipotent characteristics [7,8]. Indeed, confluent HepaRG cells supplemented with dimethyl sulfoxide (DMSO) are stimulated

Modulation Of Liver Cell Expression

Rachel Sandford



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