College of Higher Education, Educational Program for Biomedical Scientists

Mechanism of pyruvate kinase M2 uptake in liver cells

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Abstract

Treatment of liver disease relies on surgical resection of malignant tissue enabled by the liver's own ability to regenerate its lost mass. However, this is often compromised by underlying liver disease. The Pyruvate kinase (PK) M2 has been shown to be taken up by hepatocytes during liver regeneration, in the pericentral region where hypoxia is present. [1] There are two possible receptors that potentially form the entry port of PKM2; epidermal growth factor receptor (EGFR) and integrin-β 1 (ITGβ1). Using protein expression analysis of human hepatocellular carcinoma (HCC) cells, we show an increase in EGFR mainly by hypoxia. Additionally, an increase in both receptors upon incubation with human recombinant (r)PKM2. A generally increased rPKM2 uptake was observed under hypoxic conditions.

Introduction

The liver is a very metabolically active organ with multiple functions and has the unique ability to regenerate itself after tissue loss. Due to the anatomy of the liver, there is metabolic zonation in the tissue. Hepatocytes therefore have different functional and metabolic properties depending on their localization in the sinusoids. [2]

Pyruvate kinases are enzymes responsible for catalyzing the transfer of phosphate groups in the final and rate-limiting step of glycolysis. In Hepatocytes, physiologically there can be found the liver-specific L-isoform. It has been shown that hepatocytes do not produce the PK isoform M2 themselves, but that this is provided by surrounding Kupffer cells. [3]

A possible receptor for PKM2 in the liver is EGFR. Its activation plays a crucial role in the induction of cell proliferation and thus tissue growth. It has been reported that PKM2 can positively influence phosphorylation and thus activation of EGFR. [4]
The second potential receptor, ITGβ1 is responsible for cellular functions such as adhesion or repair of injured tissues and is thus partly responsible for liver regeneration. [5]

Recently, it was revealed that pyruvate kinase M2 (PKM2) plays an important role in liver regeneration. Moreover, it occurs under conditions of lower oxygen availability. Hypoxia contributes to a faster recovery of normal liver function, while diseased cells also benefit from this growth-promoting environment. [6]

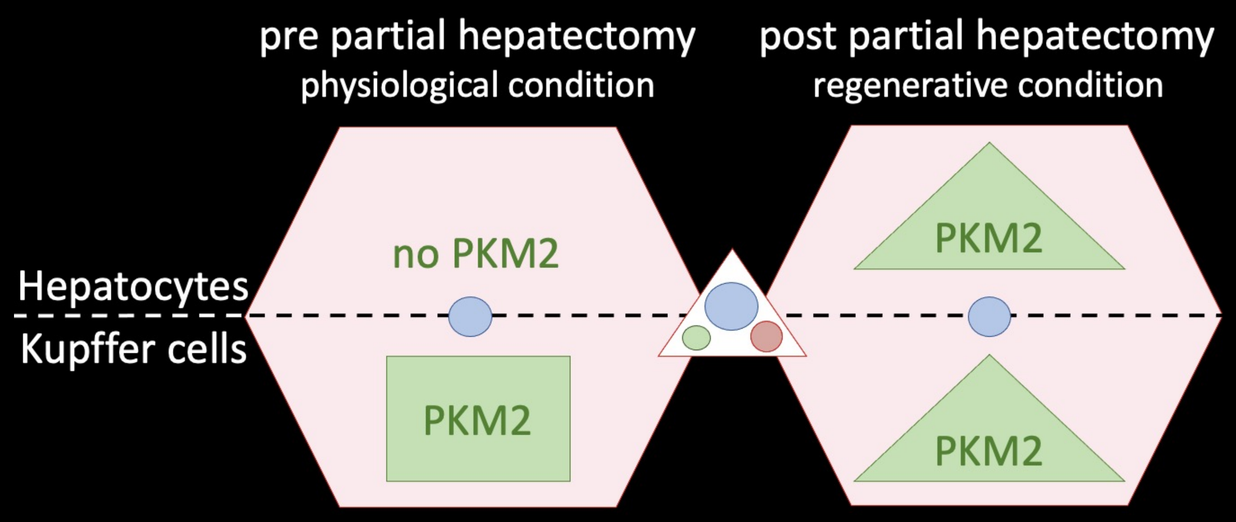

Fig. 1 *schematic representation of the zonal and quantitative distribution in the lobules of PKM2 in relation to hepatocytes and Kupffer cells.*

Aims and leading questions

The aim is to investigate the relationship between PKM2 and hypoxia in the context of liver regeneration. Specifically, this thesis wants to determine whether hypoxia affects the uptake of rPKM2 in HCC cells and whether there is an increase or decrease in EGFR or ITGβ1 activation under hypoxia and/or by adding rPKM2.

Material, methods and procedure

The utilized cell line, SNU475, was analyzed regarding its receptor expression and the uptake capacity of rPKM2 in the conditions 1.5% (hypoxia) or 21% (normoxia) oxygen.
The cells were cultivated and preconditioned overnight in either a 6-well plate for protein extraction or into a chamber slide for Immuncytochemistry (ICC). SNU475 was then incubated with rPKM2, which was produced bacterially after corresponding transformation, or control dialysate for 3 hours.

The Western blot technique (WB) with antibodies, corresponding to the receptors and their activated form, were used for protein detection. The band strength was quantified by pixel counting

In the ICC, both the rPKM2 and the receptors were detected with fluorescent antibodies. In addition, the membrane of the cells was stained for the visual localization of EGFR and ITGβ1.

Results

The following statements refer to WB as well as to ICC.
EGFR shows an increase when incubated with rPKM2 in normoxia as well as hypoxia when comparing to the control. Also, an increase from normoxia to hypoxia is recognized. In addition, an increased phosphorylation, i.e. activation, was detected.
For ITGβ1, an increasing expression and more precursor forms under hypoxia, compared to the situation in normoxia, is evident.

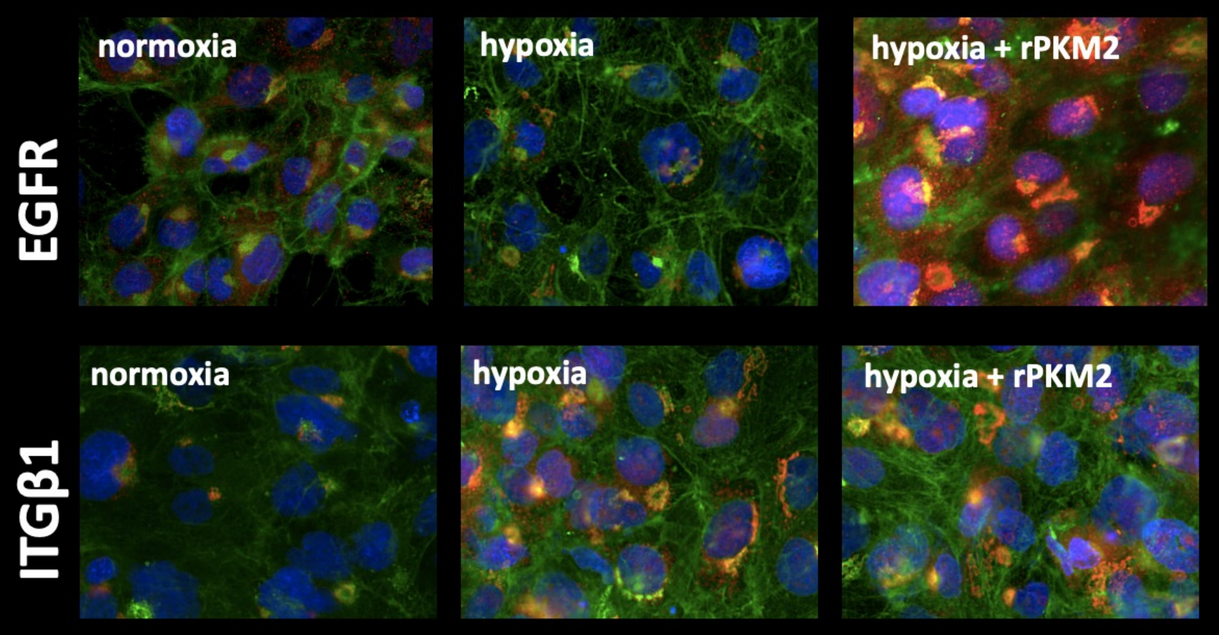

Fig. 2 *EGFR / ITGβ1 and membrane double-staining of SNU475 after 3h rPKM2 incubation. Cell nuclei are shown in blue, the membrane in green, and EGFR in red. Overlapping signals from receptor and membrane can be seen as yellow.*

Discussion

SNU475 shows a measurable response of EGFR to a situation that would be present during the process of liver regeneration. ITGβ1 is upregulated with the incubation with rPKM2 under normoxic conditions.

ITGβ1 and EGFR have been described to interact with each other. Results strengthen this link as both, ITGβ1 and EGFR show the same activation pattern upon incubation with PKM2. This suggests a connection or even a dependence of the two receptors

References

[1] Michalopoulos, G. K. (2014). Advances in liver regeneration. *Expert Review of Gastroenterology & Hepatology*, *8*(8), 897–907.
[2] Kietzmann, T. (2019). Liver Zonation in Health and Disease: Hypoxia and Hypoxia-Inducible Transcription Factors as Concert Masters. *International Journal of Molecular Sciences*, *20*(9), 2347
[3] Chatzipanagiotou, S., Nath, A., Vogt, B., & Jungermann, K. (1985). Alteration in the Capacities as well as in the Zonal and Cellular Distributions of Pyruvate Kinase L and M2 in Regenerating Rat Liver. *Biological Chemistry Hoppe-Seyler*, *366*(1), 271–280.
[4] Hsu, M.-C., Hung, W.-C., Yamaguchi, H., Lim, S.-O., Liao, H.-W., Tsai, C.-H., & Hung, M.-C. (2016). Extracellular PKM2 induces cancer proliferation by activating the EGFR signaling pathway. *Am J Cancer Res* 2016;6(3):628-638.
[5] Wang, C., Zhang, S., Liu, J., Tian, Y., Ma, B., Xu, S., Fu, Y., & Luo, Y. (2020). Secreted Pyruvate Kinase M2 Promotes Lung Cancer Metastasis through Activating the Integrin Beta1/FAK Signaling Pathway. *Cell Reports*, *30*(6), 1780-1797.e6.
[6] Gupta, V., & Bamezai, R. N. K. (2010). Human pyruvate kinase M2: A multifunctional protein: Multifunctional Human PKM2. *Protein Science*, *19*(11), 2031–2044. https

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Fig. 1 Maring Sarah (2021), *schematic representation of the zonal and quantitative distribution in the lobules of PKM2 in relation to hepatocytes and Kupffer cells. Bern*
Fig. 2 Maring Sarah (2021), *EGFR / ITG*β1 *and membrane double-staining of SNU475 after 3h rPKM2 incubation. Bern*