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REVIEW ARTICLE

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HGF/C-Met/ERK1/2 Signaling Pathway and Hepatocyte Polyploidization in Cholestatic Liver

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ABSTRACT

Background: Liver regeneration ability to restore lost or damaged tissue mass and function in response to resection, has been studied since ancient times, yet it remains a current issue in modern biology and medicine. Despite the abundance of modern experimental and clinical data, the signaling pathways that control the organ and/or individual cellular mechanisms have not yet been fully determined. Liver regeneration, which is mainly carried out by three sequential processes (proliferation, polyploidy and hypertrophy), in some pathologies (cholestatic liver), begins with polyploidization of parenchymal cells, although the specific signaling pathway that initiates it, hasn't yet been established. For example, the key signaling pathway HGF/C-Met/ERK1/2 responsible for proliferation and differentiation, whether or not involved in the process of polyploidization. The aim of the presented study was to investigate the role of the ERK1/2 signaling pathway in the process of polyploid cell formation in cholestatic liver.

Research Objects and Materials: Experiments were conducted on adult white rats using a cholestatic liver model induced by CBDL. Experiment was performed by using C-Met receptor inhibitor - PHA665752 and MEK1/2 signaling pathway inhibitor - PD98059.

Methods: Proliferative activity was assessed via the colchicine mitotic index, and changes in hepatocyte ploidy were analyzed using Feulgen staining and ImageJ software.

Results: Inhibition of the HGF receptor significantly reduced hepatocyte mitotic activity in cholestatic rats, but didn't affect polyploidization, suggesting that liver responds to functional demands by activating the well-known HGF/C-Met/ERK1/2 signaling pathway cascade to stimulate proliferation. In contrast, inhibition of MEK1/2 in cholestatic animals results increase in diploid (2c) cells, decrease in binucleated tetraploid (2c×2) cells and no mononuclear or binuclear octoploid (8c, 4c×2) cells weren't observed.

Conclusion: At the initial stage of cholestasis induced by common bile duct ligation, the liver responds to increased functional demands, without mass loss, through hepatocyte polyploidization. This process is initiated by the activation of the Tpl2-MEK-ERK signaling pathway.

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Cholestatic Liver, Polyploidization, Regeneration, ERK1/2 Signaling Pathway

Introduction

Along with many vital functions, the liver, as is well known, has a higher regenerative capacity compared to other organs. Nevertheless, the molecular and cellular mechanisms of regeneration of the significant glandular organ of vertebrates and humans have not yet been fully determined, and therefore, it still remains a subject of active research. The knowledge of these mechanisms is of immense importance for modern clinical medicine, as it has been established that during various diseases, the organ's ability to regenerate is not always sufficient for the full restoration of its functions. The initiation of new research in the field of liver regeneration is also driven by the existence of recent, yet contradictory scientific data in some cases. For example, it is considered established that liver regeneration occurs through three main processes: proliferation, polyploidy, and hypertrophy [1,2]. The regulation of these processes is carried out through several signaling pathways, the activation of which is initiated by various growth factors [3,4]. Recent studies, however, have yielded relatively limited information indicating that organ regeneration does not always occur simultaneously through all three aforementioned mechanisms. For example, in the model of alimentary dyslipidemia, it has been shown that the mechanism through which organ regeneration occurs depends on the duration of the hepatogenic diet and the extent of the damage. Specifically, it has been shown that, at the initial stage, tissue regeneration is primarily achieved through an increase in the ploidy of hepatic parenchymal cells [5]. Through polyploidization, without an increase in the number of cells, the regeneration process also occurs at the initial stage of regenerative growth in cholestatic liver of rats [6]. In response to trauma, an increase in DNA ploidy has also been described in the tissues of various organs in mammals (heart, liver, kidney) [7]. It is also noteworthy that after liver resection, the blockade of mitosis caused by the inactivation of cyclin-dependent kinase 1 does not impair the restoration of liver function and proceeds via polyploidization, which indicates

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that regeneration in this case occurs without cell division [8].

Based on the above, it can be concluded that in some pathologies, liver regeneration begins with the polyploidization of parenchymal cells. At the same time, it is still not fully understood whether, during different liver pathologies, the same signaling pathway(s) contribute to the synchronized growth of the genome. During regeneration, mitogen-activated protein kinases (MAPKs) play a significant role. Through their involvement, cascade reactions of phosphorylation mediated in the liver by 4 signaling pathways (ERK1/2, JNK1/2/3, p38, and ERK5) are initiated by various growth factors. Among them, a leading role is attributed to hepatocyte growth factor (HGF), who's binding to the C-Met receptor in the liver activates the ERK1/2 signaling pathway. It has been shown that during cholestasis induced by the obstruction of the common bile duct, the levels of HGF and c-Met mRNA in the liver increase [9-11]. At the same time, it was shown by us that on the second day after the obstruction of the common bile duct, the ploidy of hepatocytes increases in cholestatic liver [12]. Moreover, the MEK1/2 and consequently ERK1/2 signaling pathway can be activated in response to inflammatory processes, regulated by TPL2, which has been shown to activate p38α and p38δ in neutrophils. [13].

Based on the above, the aim of the present study was to investigate the role of the ERK 1/2 signaling pathway in the process of polyploid cell formation in an experimental cholestatic liver model.

Materials and Methods

Experiments were carried out on adult (130-150 g) white rats. Model of cholestatic liver with common bile duct ligation (CBDL) was used. Animals were housed under controlled conditions at a temperature of 25 ± 2˚C, relative humidity of 60 ± 10%, with room air changes 12-18 times/hour, and a dark/light ratio=14/10. During the experiment, they were provided with unrestricted access to water and food.

To determine the involvement of the ERK1/2 signaling pathway in hepatocyte polyploidization in cholestatic liver, we performed the experimental work in two stages. In the first stage, we blocked the HGF receptor (C-Met). For this purpose, the animals were divided into three groups (10 animals per group): I. Control group – intact animals, II. First experimental group – animals subjected to common bile duct ligation (4 days), III. Second experimental group – animals subjected to common bile duct ligation and then intraperitoneally treated with the HGF C-Met receptor inhibitor (PHA665752) (1 mg/kg) during surgery and for the next three days, once a day (total of 4 doses). Tissue samples were collected on the fourth day after operation.

In the second stage, we directly inhibited MEK1/2 in the cytoplasm. The animals were divided into three groups (10 animals per group): I. Control group – intact rats, II. First experimental group - animals 48 hours after common bile duct ligation (CBDL), III. Second experimental group - animals that received an intraperitoneal injection of the MEK1/2 inhibitor (PD98059) (10 mg/kg) 20 hours after common bile duct ligation.

The proliferative activity of liver tissue was assessed using colchicine mitotic index determination method (1 mg/kg colchicine injected into rats). Liver smears were stained with Feulgen stain for DNA-specific staining, followed by microscopic examination. Images were analyzed using the ImageJ software.

Results

In the first series of experiments, as mentioned above, we initially assessed the changes in the hepatic proliferative activity of the animals in the first experimental group on the 4th day after CBDL. In figure 1, histograms showing the changes in mitotic activity of the control and two experimental groups of animals are presented. The analysis of these histograms showed that, compared to the control group animals, the mitotic activity of hepatocytes in the rats of the first experimental group significantly increased, reaching 15‰. At the same time point, as shown in figure 1, in the animals of the second experimental group, which received injections of the HGF C-Met receptor inhibitor (PHA665752) (one injection per day for 4 days), the mitotic activity was statistically significantly reduced compared to the corresponding values of the first experimental group. Specifically, the colchicine mitotic index was decreased by approximately 50% (Figure 1, Figure 2 B, C).

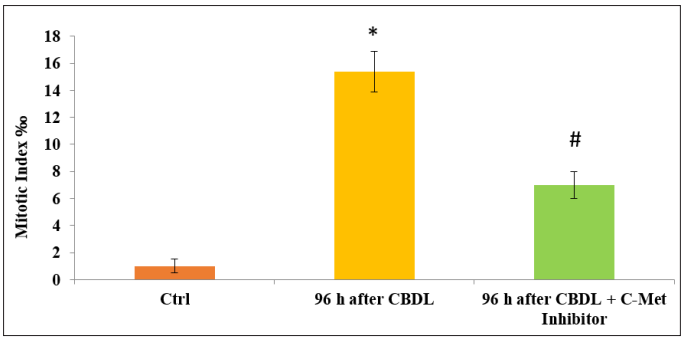

Figure 1: Effect of C-Met inhibitor on changes in mitotic activity of cells in cholestatic liver of adult white rats.

*P < 0.05 Significant increase compared to control.

#P < 0.05 Significant decrease compared to 96 h after CBDL animal group.

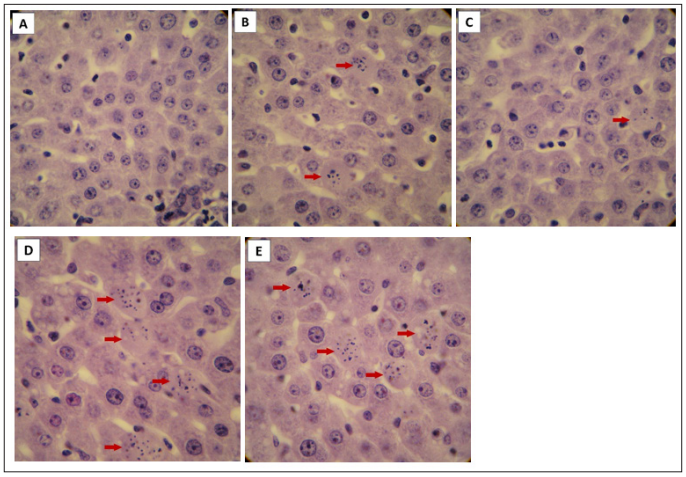

Figure 2: The Liver Tissue Histoarchitecture of Control (Intact) and Experimental Animal Groups. H&E. Intact group A: 96 h after CBDL, B: 96 h after CBDL+C-Met inhibitor, C: 48 h after CBDL, D: 48 h after CBDL+ ERK1/2 inhibitor, E: (A, B, C, D, E x 400). Arrows indicate mitotic cells.

The significant reduction in the hepatocyte mitotic index in response to injections of the C-Met receptor inhibitor under cholestatic conditions suggests that, even under cholestasis, the liver responds to functional demands by activating the well-known HGF/C-Met/ERK1/2 signaling pathway cascade to stimulate proliferation. At the same time, it is still unknown whether the liver uses well-studied adaptive mechanisms, such

as polyploidization, under these conditions. Therefore, in this series of experiments, we investigated the quantitative changes in the number of polyploid cells in the liver of animals from all three groups described above. The studies revealed that, on the 4th day after bile duct ligation in the first experimental group of rats, the number of diploid cells in the liver decreased by approximately half. At the same time, the number of tetraploid (4c) cells increased twofold, and the number of octaploid hepatocytes (8c) also increased slightly, but significantly (Figure 3). Based on the analysis of the obtained data, it can be hypothesized that the HGF/C-Met/ERK1/2 signaling pathway is not involved in the initiation of polyploidization. According to the literature, the MEK/ERK signaling pathway plays an important role in the regulation of various vital processes [14,15]. However, the role of the HGF-activated MEK/ERK1/2 signaling pathway in hepatocyte polyploidization during cholestasis remains unknown.

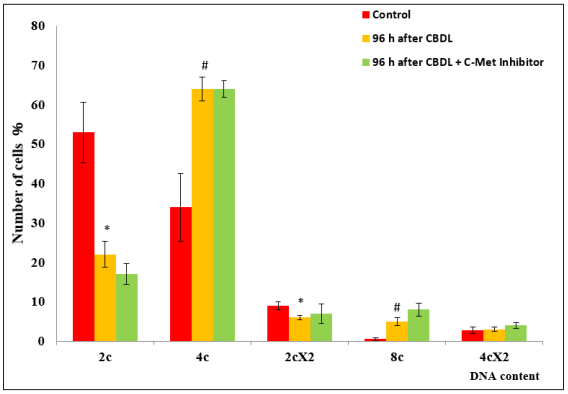

Figure 3: Effect of C-Met Inhibitor on Changes in Hepatocyte Ploidy in Cholestatic Liver of Adult White Rats.

*P < 0.05 Significant decrease compared to control group.

#P < 0.05 Significant increase compared to control group.

To verify the proposed hypothesis, in the second series of experiments, we directly inhibited MEK1/2 in the cytoplasm using the inhibitor PD98059 (10 mg/kg). In this case, we initially assessed the changes in hepatocyte proliferative activity in cholestatic liver by determining the colchicine mitotic index. The obtained results are presented in Figure 4.

As seen in the figure, in the first experimental group of rats, 48 hours after ligation of the common bile duct, mitotic activity significantly increased compared to the control group animals (Figure 2. D, E). Furthermore, in the second experimental group, where the MEK1/2 inhibitor was administered 20 hours after bile duct ligation, no significant changes in the number of mitotic cells were observed (Figure 4).

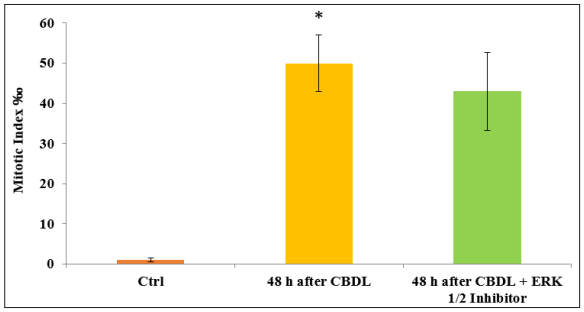

Figure 4: Effect of MEK 1/2 Inhibitor on Changes in Mitotic Activity of Cells in Cholestatic Liver on 48 Hours after Ligation of the Common Bile Duct.

*P < 0.05 Significant Increase Compared to Control

In this second series of experiments, we also assessed the effect of the MEK 1/2 inhibitor on changes in the quantitative ratio of polyploid hepatocytes in cholestatic liver. It was found that, under the influence of this inhibitor, the number of tetraploid cells increased compared to the control group. Notably, the increase in polyploid cells occurred at the expense of octaploid, mononuclear, and binuclear hepatocytes. In the third experimental group, there was an increase in diploid (2c) cells. A trend toward a decrease in binucleated tetraploid (2c×2) cells was observed. Additionally, no mononuclear or binuclear octaploid (8c, 4c×2) cells were observed at all (Figure 5).

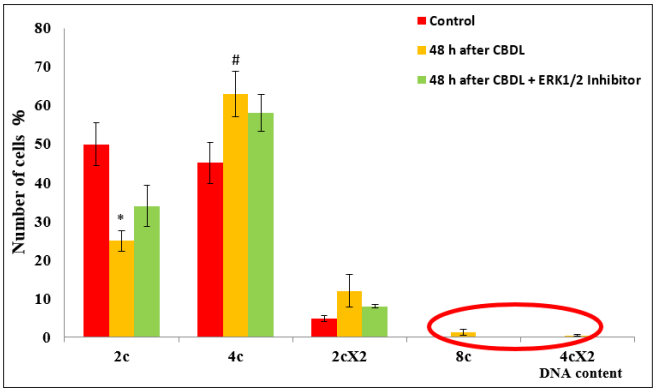

Figure 5: Effect of MEK 1/2 Inhibitor on Changes in the Quantitative Ratio of Polyploid Cells in Cholestatic Liver of Adult White Rats.

*P < 0.05 Significant decrease compared to control group.

#P < 0.05 Significant increase compared to control group.

Discussion

The formation of polyploid cells in the liver of mammalian is considered one of the mechanisms for function restoration and adaptation in response to mass loss or injury, along with cell proliferation and hypertrophy. The regulation of these processes occurs through several signaling pathways, whose activation is initiated by different growth factors [3,4]. The signaling pathways driving liver regeneration and their molecular activation mechanisms are well studied [16]. However, there is relatively limited data regarding the fact that, in some pathologies, without mass loss, the liver responds to increased functional demands at the early stages by an increase in the number of polyploid cells [17]. Moreover, according to recent data, polyploidization is considered one of the mechanisms for function restoration in response to liver injury, which occurs at the initial stage of liver regeneration in certain pathologies. In one pathology, specifically on the second day after bile duct ligation, there is an increase in hepatocyte ploidy in cholestatic liver [3-7,12]. Additionally, during cholestasis induced by common bile duct ligation, the HGF level in the liver increases [18]. The binding of HGF to the C-Met receptor, in turn, triggers the activation of the RAS-RAF-MEK-ERK signaling pathway [19]. The MEK/ERK signaling pathway plays a significant role in cell proliferation, differentiation, and the regulation of various physiological processes. Therefore, its dysregulation is often associated with the development of cancer cell growth [14,15,20]. However, the role of the HGF-activated MEK/ERK signaling pathway in hepatocyte polyploidization during cholestasis remains unknown.

Based on the data obtained from the cholestatic liver of white rats, where the c-Met receptor was blocked using the PHA665752 inhibitor, the reduced hepatocyte proliferation and unchanged polyploidization allow us to hypothesize that the HGF/C-Met signaling pathway has the potential to contribute to liver regeneration through the stimulation of hepatocyte proliferation,

but does not participate in the polyploidization of cholestatic liver cells.

The binding of HGF to the c-Met receptor regulates the MEK/ERK signaling pathway, which is also regulated by the major inflammatory mediator TPL2 [13,19]. Given that strong inflammatory processes occur in the liver in response to common bile duct ligation [10], it is possible that the Tpl2-MEK-ERK signaling pathway could be activated, leading to MEK/ERK activation even under conditions of c-Met receptor blockade. Considering all of this, it is possible that the activation of ERK 1/2 could initiate polyploidization in cholestatic liver. Our hypothesis was confirmed by an experiment in which we used the MEK 1/2 inhibitor (PD98059). As a result of using this inhibitor, it was found that in the liver of rats from the second experimental group, octaploid mononuclear and binuclear hepatocytes no longer increased. The obtained results suggest that the MEK/ERK signaling pathway is involved in the initiation of polyploid hepatocyte formation under cholestatic conditions.

Along with the MEK/ERK signaling pathway, the c-Met receptor also regulates the PI3K/Akt signaling pathway, and even under conditions of PI3K/Akt pathway inactivation, a reduction in the formation of binucleated tetraploid (2c×2) hepatocytes is observed [21-23]. This indicates that the PI3K/Akt signaling pathway regulates the formation of binucleated tetraploid (2c×2) hepatocytes.

Therefore, the trend of reduction (non-significant) in 2c×2 cells observed in our study under MEK/ERK pathway inhibition may have been driven by the activation of the PI3K/Akt signaling pathway.

Conclusion

The results of our study indicate that, at the initial stage of cholestasis induced by common bile duct ligation, the liver responds to increased functional demands, without mass loss, through hepatocyte polyploidization. This process is initiated by the activation of the Tpl2-MEK-ERK signaling pathway.

References

[1] Than NN, Newsome PN. Stem cells for liver regeneration. An International Journal of Medicine. 2014; 107: 417-421.

[2] Sakuta GA, Kudriavtsev BN. Cellular mechanisms of cirrhotic rat liver regeneration. II. Proliferation polyploidization and hypertrophy after partial hepatectomy. Tsitologia. 2005; 47: 379-387.

[3] Melanie Rodrigues, Linda G Griffith, Alan Wells. Growth factor regulation of proliferation and survival of multipotential stromal cells. Stem Cell Research & Therapy. 2010; 1: 32.

[4] Debopriya Choudhury, Dhruba Ghosh, Meghna Mondal, Didhiti Singha, Ramesh Pothuraju, et al. Polyploidy and mTOR signaling: a possible molecular link. Cell Communication and Signaling. 2024; 22: 196.

[5] Bivalkevich NV, Karaman Yu K. Mechanisms of rat liver regeneration in alimentary dyslipidemia. Health Medical Ecology Science. 2009; 4: 39-40.

[6] Bakuradze E. Charachteristic of liver regeneration in codition of cholestasis (Ph.D. Thesis). Tbilisi, Georgia. 2006; https://rustaveli.org.ge/res/docs/e5bce008b2a3d053a9a6d24b12349da3602e0f4e.pdf.

[7] Erin C Bailey, Sara Kobielski, John Park, Vicki P Losick. Polyploidy in Tissue Repair and Regeneration. Cold Spring Harb Perspect Biol. 2021; https://cshperspectives.cshlp.org/content/13/10/a040881.

[8] Kasim Diril M, Chandrahas Koumar Ratnacaram, Padmakumar VC, Tiehua Duc, Martin Wasser, et al. Cyclin-dependent kinase 1 (Cdk1) is essential for cell division and suppression of DNA re-replication but not for liver regeneration. Proc Natl Acad Sci. 2012; 109: 3826-3831.

[9] Gabrielle Westenberger, Jacob Sellers, Savanie Fernando, Sadie Junkins, Sung Min Han, et al. Function of Mitogen-Activated Protein Kinases in Hepatic Inflammation. J Cell Signal. 2021; 2: 172-180.

[10] Yoon Seok Roh, Ara Cho, Youn-Soo Cha, Suk-Heung Oh, Chae Woong Lim, et al. Lactobacillus Aggravate Bile Duct Ligation-Induced Liver Inflammation and Fibrosis in Mice Toxicol. Res. 2018; 34: 241-247

[11] Emiko Uchikawa, Zhiming Chen, Guan-Yu Xiao, Xuewu Zhang, Xiao-chen Bai. Structural basis of the activation of c-MET receptor. Nature Communications. 2021; 12: 4074.

[12] Salome Kiparoidze, Nino Karumidze, Ekaterine Bakuradze, Irina Modebadze, Levan Rusishvili, et al. Study of Hepatocytes Polyploidization Peculiarities in Cholestatic Liver of Adult Rats. MCB. 2021; 18: 131-138.

[13] Daqi Xu, Marissa L Matsumoto, Brent S McKenzie, Ali A Zarrin. TPL2 kinase action and control of inflammation. Pharmacological Research. 2018; 129: 188-193.

[14] Renee Barbosa, Lucila A Acevedo, Ronen Marmorstein (2021) The MEK/ERK Network as a Therapeutic Target in Human Cancer. Mol Cancer Res 19: 361-374.

[15] Elmira Roshani, Mohammad Amini, Souzan Najafi, Behzad Mansoori, Ahad Mokhtarzadeh, et al. Interplay between MAPK/ERK signaling pathway and MicroRNAs: A crucial mechanism regulating cancer cell metabolism and tumor progression. Life Sciences. 2021; 278: 119499.

[16] Xiao Ma, Tengda Huang, Xiangzheng Chen, Qian Li, Mingheng Liao, et al. Molecular mechanisms in liver repair and regeneration: from physiology to therapeutics. Signal Transduction and Targeted Therapy. 2025; 10: 63.

[17] Diana Dzidziguri, Ekaterine Bakuradze, Irina Modebadze, Merab Sepashvili, Giorgi Aleksanashvili, et al. The Adaptive Mechanism of Liver in Response to Hyperglycemic Functional Stress in Alloxan Induced Diabetes. PROGRESS IN MEDICAL SCIENCES. 2024; 8: 1-3.

[18] Salas-Silva S, López-Ramirez J, Barrera-Chimal J, Lazzarini-Lechuga R, Simoni-Nieves A, et al. Hepatocyte growth factor reverses cholemic nephropathy associated with α-naphthylisothiocyanate- induced cholestasis in mice. Life Sci. 2022; 295: 120423.

[19] Yazhuo Zhang, Mengfang Xia, Ke Jin, Shufei Wang, Hang Wei, et al. Function of the c-Met receptor tyrosine kinase in carcinogenesis and associated therapeutic opportunities. Mol Cancer. 2018; https://molecular-cancer.biomedcentral.com/counter/pdf/10.1186/s12943-018-0796-y.pdf.

[20] C Fremin, S Meloche. From basic research to clinical development of MEK1/2 inhibitors for cancer therapy. Journal of Hematology and Oncology. 2010; 3: 2-11.

[21] Peter V Usatyuk, Panfeng Fu, Vijay Mohan, Yulia Epshtein, Jeffrey R, et al. Role of c-Met/Phosphatidylinositol 3-Kinase (PI3k)/Akt Signaling in Hepatocyte Growth Factor (HGF)-mediated Lamellipodia Formation, Reactive Oxygen Species (ROS) Generation, and Motility of Lung Endothelial Cells. the journal of biological chemistry. 2014; 289: 13476-91.

[22] Xiaodong Chen, Fen Tian, Peng Lun, Yugong Feng. Curcumin Inhibits HGF-Induced EMT by Regulatingc-MET-Dependent PI3K/Akt/mTOR SignalingPathways in Meningioma. Evidence-Based Complementary and Alternative Medicine 2021: 10.

[23] Séverine Celton-Morizur, Grégory Merlen, Dominique Couton, Chantal Desdouets. Polyploidy and liver proliferation: central role of insulin signaling; Cell Cycle. 2010; 9: 460-466.