Using AI and Bioinformatics tools to predict the hypothetical mechanism of USAG1 gene in regeneration of human liver

Ahmed Mohamed Abdelmonaam Ali 20181018

Ahmed Ebrahim El-metwally Ahmed 20191003

Mohamed Abd Al-Moneam Mohamed El Attar 20191124

Under the supervision of Prof Dr Hatem Elkordy

Introduction:

The field of regenerative medicine has made tremendous strides in recent years, offering hope for the restoration of damaged tissues and organs. One of the most exciting areas of research is the application of gene therapy to enhance tissue regeneration, particularly in the context of complex organs like the liver and teeth. The liver, known for its remarkable regenerative capacity, serves as an invaluable model for understanding the mechanisms that govern tissue repair and regeneration.

In this project, we explore the possibility of using liver regeneration as a simulation model for tooth regeneration, with a focus on gene therapy as a tool to induce regenerative processes.

The therapeutic potential of gene therapy lies in its ability to precisely modulate key genes involved in tissue regeneration.

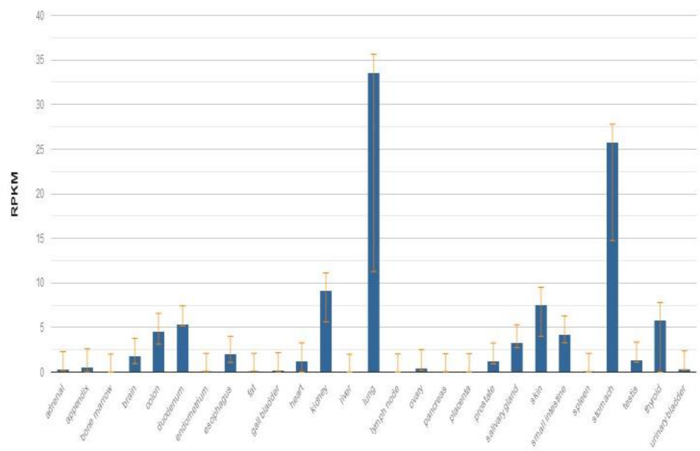
This research aims to expand our understanding of regenerative mechanisms and establish a proof-of-concept approach for liver regeneration using gene therapy, with the long-term goal of translating these insights into clinically viable therapies.

Uterine sensitization-associated gene-1 or usag1 gene

Gene ID: 25928

It is also called Sostdc1/CDA019/DAND7/ECTODIN

it is an important gene in number of signaling pathways in the body it encodes a protein that is involved in number of cellular processes and organ regeneration



The USAG1 gene is located on chromosome 17 at position 17p13.1.

It has 2 Introns and 2 exons

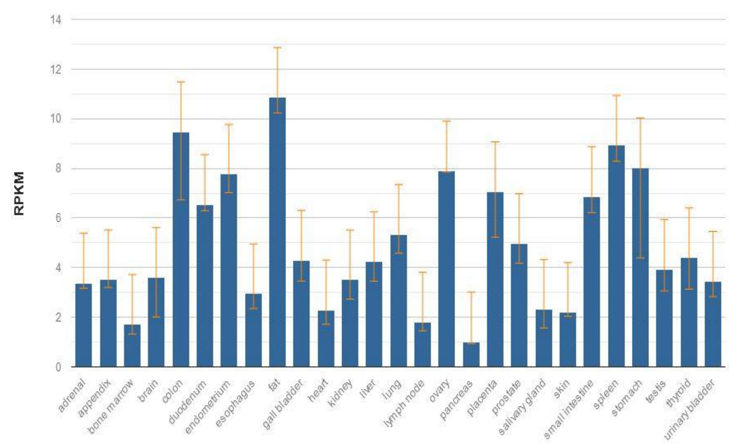
This gene act in the time frame of early first 6 hours.

transcription factor 7 like 2 (TCF7L2)

Gene ID: 6934

It is also called TCF4; TCF-4

It plays a key role in the Wnt/β-catenin signaling pathway, which is crucial for various biological processes, including liver regeneration.



TCF7L2 is located on chromosome 12 (specifically at 12q13.2).

It has 15 exons and 14 introns

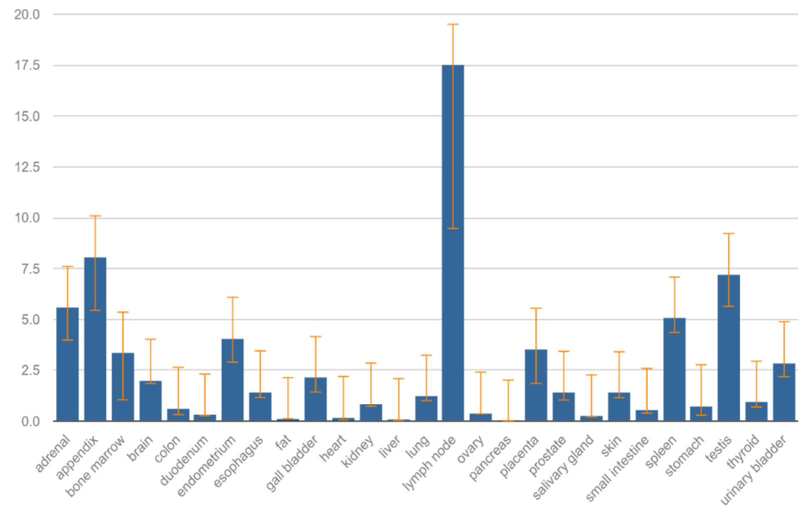
contributes to the maintenance and renewal of stem cells in the liver

act on the time frame of 12-48 hours.

lymphoid enhancer binding factor 1 (LEF1)

Gene ID: 51176

It is also called LEF-1 / TCF10/ TCF7L3/ TCF1ALPHA



it promotes hepatocyte proliferation by facilitating the activation of genes that control the cell cycle.

It is located on chromosome 4 (4q25)

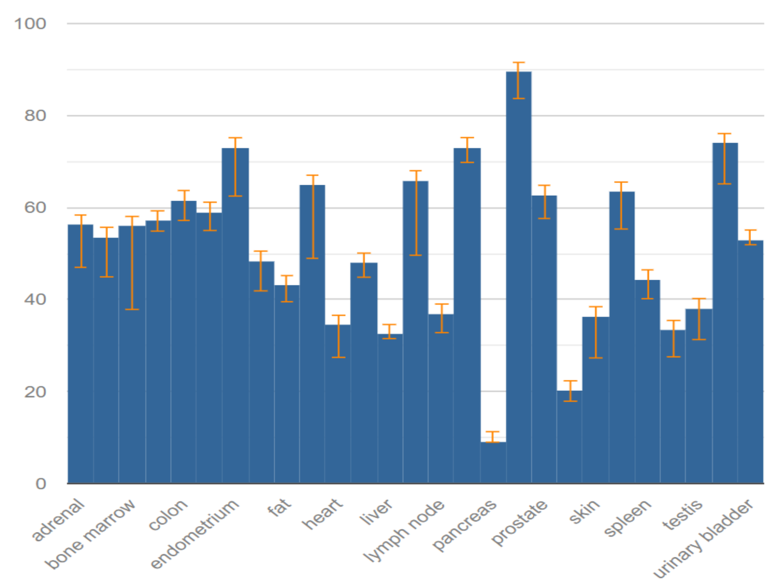
it has 4 introns and 5 introns

it works at time frame of 12-48 hours.

catenin beta 1 CTNNB1 (β-catenin)

Gene ID: 1499

it is also called EVR7; CTNNB; MRD19; NEDSDV



it helps the regenerative response after liver injury or partial hepatectomy

The gene is in chromosome 3 at position 3p21.31

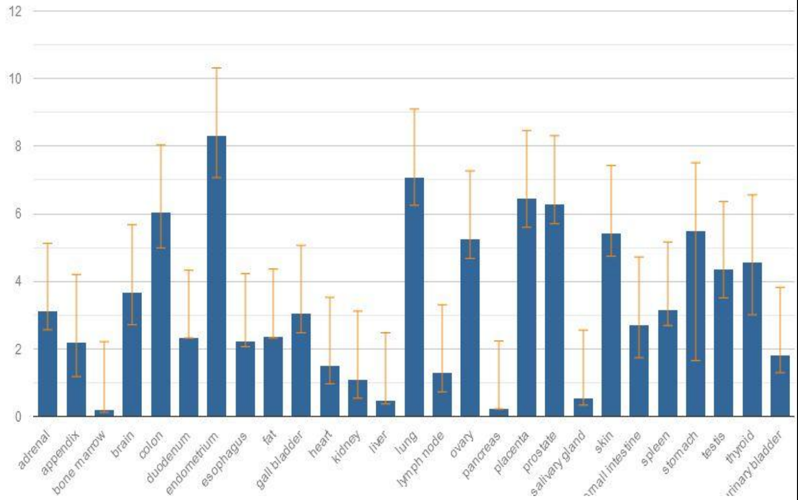
It has 14 introns and 15 exons

It works in time frame 12-48 hours.

AXIN2

Gene ID: 8313

it is also called AXIL; ODCRCS



AXIN2 helps maintain the proper balance of Wnt signaling, ensuring effective tissue repair and regeneration after injury or partial hepatectomy

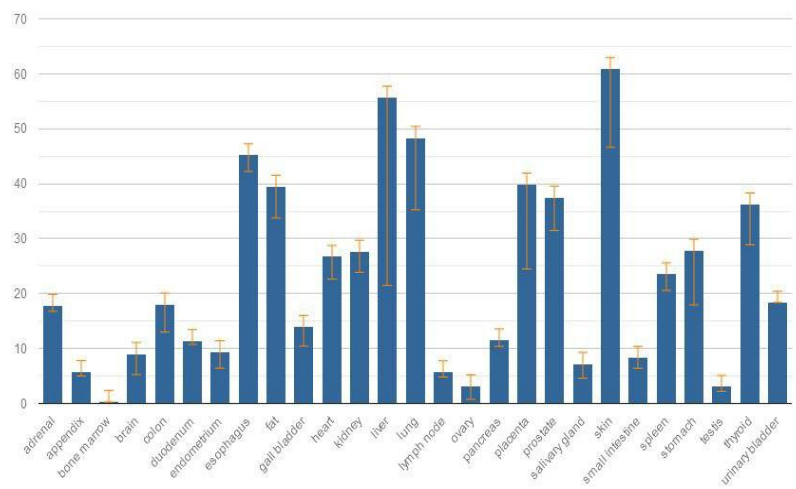
it is located on chromosome 17 at position 17q24.3.

It has 14 introns and 15 exons.

It works at time frame of Early to Mid (12–48 hours).

Cyclin D1 (CCND1)

Gene ID: 595



It is also called BCL1; PRAD1; U21B31

The CCND1 gene regulates the cell cycle by promoting the transition from the G1 phase to the S phase

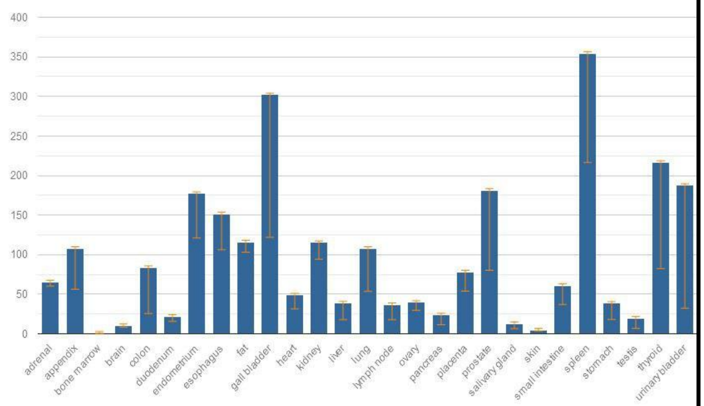
It is located on chromosome 11 at position 11q13.3.

it has 4 introns and 5 exons

it works at time frame of 48-72 hours.

Cellular communication network factor 2 (CCN2)

Gene ID: 1490



It is also called CTGF; NOV2; HCS24; IBP-8; IGFBP8

plays a significant role in tissue repair, fibrosis, and regeneration. CCN2 is involved in various biological processes such as cell adhesion, proliferation, differentiation, and survival.

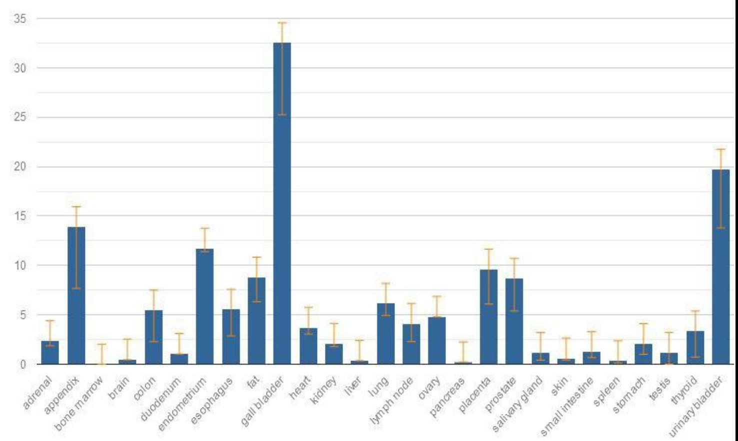
It is located at chromosome 6 at position 6q23.2.

It has 5 introns and 6 exons

It works at time frame of 48-96 hours.

fibroblast Growth Factor 7 (FGF7)

Gene ID: 2252



It is also called KGF; HBGF-7

plays a crucial role in cell growth, differentiation, and tissue repair.

It is located on chromosome 15 at position 15q22.33.

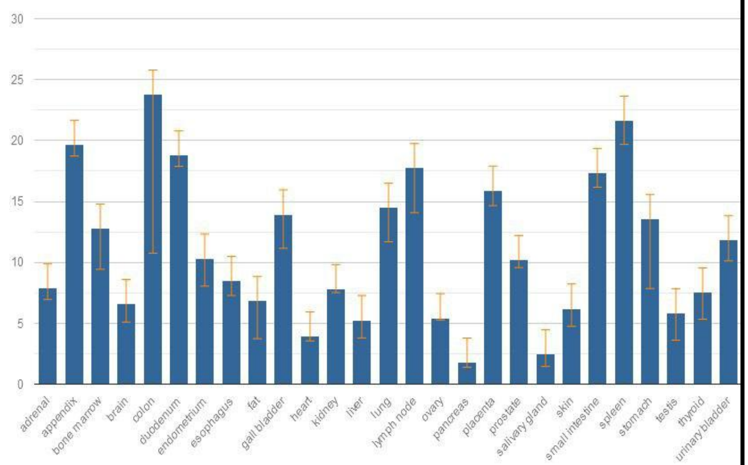
It has 5 introns and 6 exons

it works at time frame of Mid to Late (48– 96 hours).

BCL-2 Associated X Protein (BAX)

Gene ID: 581

it is also called BCL2L4



It promotes cell death by inducing mitochondrial outer membrane permeabilization. In the context of liver regeneration

it is located on chromosome 19 at position 19q13.32.

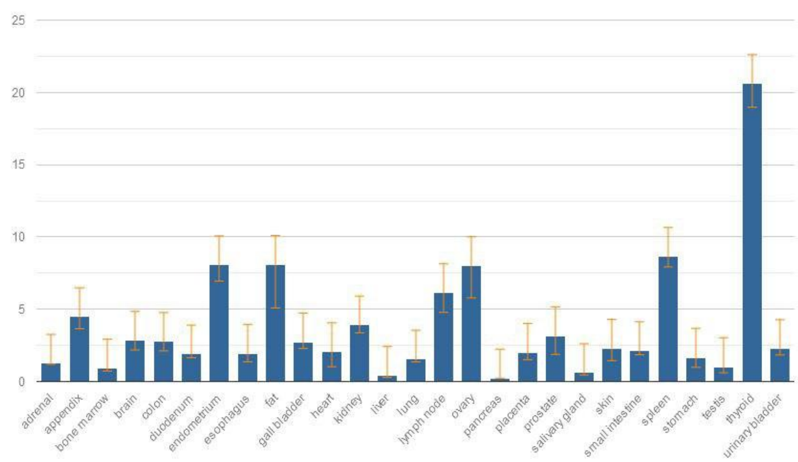
it has 2 introns and 2 exons

it works at time frame of Mid to Late (48–96 hours).

cyclin-dependent kinase 2 (CDK2)

Gene ID: 1017

It is also called CDKN2; p33(CDK2)



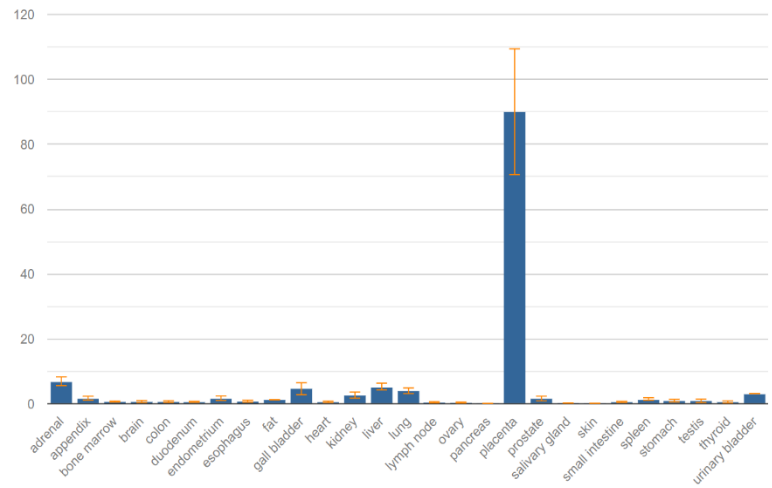
is a crucial protein kinase involved in regulating the cell cycle. It plays a significant role in controlling the G1 to S phase transition, making it a key player in cell proliferation and DNA replication.

It is located on chromosome 12 at position 12p13.2.

It has 10 introns and 11 exons

It works at time frame of Mid (48–72 hours).

Hepatocyte growth factor (HGF)



Gene ID: 3082

It is also called SF; HGFB; HPTA; F-TCF; DFNB39.

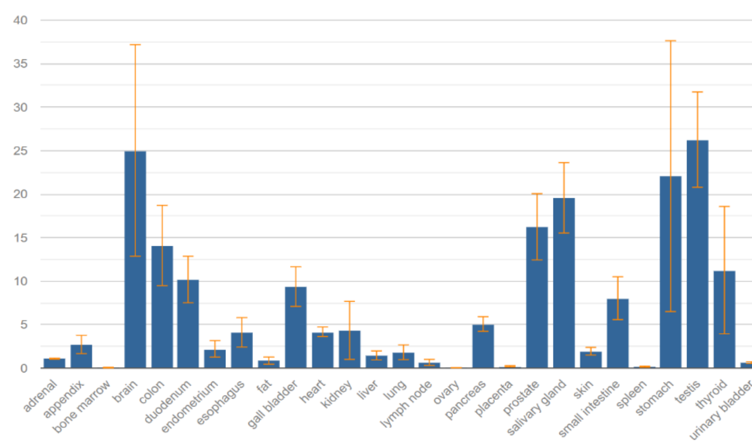
It plays a pivotal role in liver regeneration, as well as in processes like cell proliferation, migration, differentiation, and tissue repair

It is located on chromosome 7 at position 7q21.1

It has 12 introns and 13 exons

It works at time frame of Mid to Late (48–96 hours).

SRY-box transcription factor 9 (SOX9)



Gene ID: 6662

it is also called TES; CMD1; SRA1; CMPD1; ENH13; SRXX2; TESCO; SRXY10

it plays a key role in cell differentiation and regeneration. While it is primarily known for its roles in cartilage formation.

It is located on chromosome at position 17q24.3.

It has 1 intron and 3 exons.

It works at time frame of Mid (48–72 hours).

Methodology :

We asked the AI the following questions about how to regenerate liver as a simulation of the Japanese project of regenerating teeth using usag1 gene

1.predict which genes could interfere with usag1 to regenerate liver

The AI answer

1. CTNNB1 (β-catenin)
2. YAP1
3. CDKN1A
4. LGR5
5. HGF
6. EGF
7. AXIN2
8. MYC
9. Notch1

Inhibiting USAG1 could stimulate liver regeneration by activating key pathways. CTNNB1 (β-catenin) would enhance Wnt signaling, promoting hepatocyte proliferation and liver stem cell activation. YAP1 would be activated by inhibiting the Hippo pathway, promoting cell survival and regeneration. CDKN1A would be downregulated, allowing for cell cycle progression and hepatocyte division. LGR5 could activate liver progenitor cells, contributing to tissue regeneration. Growth factors like HGF and EGF would be upregulated, driving hepatocyte growth. AXIN2 could be modulated to further stabilize Wnt signaling, enhancing liver repair. MYC would drive cell proliferation, promoting rapid tissue recovery. NOTCH1 signaling would activate liver progenitor cell differentiation, completing the regenerative process.

2.using all these genes try to postulate and predict a mechanism to regenerate liver

1. USAG1: USAG1 is a negative regulator of tissue regeneration, likely inhibiting pathways like Wnt/β-catenin. Inhibiting USAG1 can enhance liver regeneration by promoting hepatocyte proliferation and progenitor cell activation. Its regulation of various signaling pathways is crucial for modulating liver repair.
2. BMP7: BMP7 (Bone Morphogenetic Protein 7) promotes hepatocyte proliferation and protects against liver fibrosis. It is involved in tissue regeneration by activating liver progenitor cells and improving cellular differentiation. BMP7 signaling is essential for liver repair and recovery after injury.
3. BMP4: BMP4 is involved in regulating the differentiation of liver progenitor cells and maintaining liver homeostasis. It activates specific signaling pathways that drive hepatocyte growth and regeneration. BMP4's role is critical in enhancing liver repair and stem cell differentiation during regeneration.
4. CTNNB1 (β-catenin): β-catenin is a central effector in the Wnt signaling pathway, promoting hepatocyte proliferation and liver stem cell activation. Activation of β-catenin enhances liver regeneration by promoting cell growth and survival. It is crucial for maintaining regenerative capacity in the liver.
5. AXIN2: AXIN2 is a key regulator in the Wnt/β-catenin pathway, acting as a negative regulator to control β-catenin degradation. Modulating AXIN2 can influence liver regeneration by either promoting or inhibiting Wnt signaling. Disrupting its function may enhance regeneration by stabilizing β-catenin activity.
6. TGFB1: TGF-β1 (Transforming Growth Factor β1) plays a crucial role in fibrosis and cell growth regulation. While it can promote fibrosis in the liver, inhibiting TGF-β1 signaling may alleviate scarring and foster liver regeneration. Reducing TGF-β1's activity is important for reversing liver damage and promoting repair.

7. BAX8: BAX8 is a pro-apoptotic gene that plays a role in regulating cell death, specifically in liver cells. It is involved in the apoptotic process and can inhibit excessive cell survival during liver regeneration. Modulating BAX8 expression could help balance apoptosis and regeneration during liver injury.
8. BAX2: Like BAX8, it regulates apoptosis and maintains tissue homeostasis. In the context of liver regeneration, it helps prevent excessive cell survival by promoting cell death when necessary. Inhibiting BAX2 could enhance hepatocyte survival, promoting regeneration after liver damage.
9. FGF2: FGF2 (Fibroblast Growth Factor 2) is crucial for stimulating hepatocyte proliferation and liver tissue repair. It promotes liver regeneration by activating pathways that encourage cell survival and growth. FGF2 plays an essential role in enhancing liver recovery after injury or disease.
10. MYC: MYC is a potent oncogene that drives cell cycle progression and proliferation. It plays a critical role in liver regeneration by promoting the rapid division of hepatocytes and progenitor cells. Activation of MYC can enhance liver recovery by increasing cellular turnover and tissue repair.
11. NOTCH1: NOTCH1 signaling regulates liver progenitor cell differentiation and maintains stem cell populations. It controls the fate of cells within the liver, promoting the generation of new hepatocytes. Activating NOTCH1 can help differentiate progenitor cells into functional liver cells during regeneration.
12. FOXO1: FOXO1 is a transcription factor that regulates cell survival, stress resistance, and apoptosis. It promotes liver regeneration by protecting hepatocytes from oxidative stress and facilitating recovery after injury. FOXO1’s role in managing cell survival and damage response is key to effective liver repair.
13. YAP1: YAP1 (Yes-associated protein) is a critical regulator in the Hippo signaling pathway, promoting cell proliferation and survival. In liver regeneration, YAP1 activation enhances hepatocyte growth and tissue repair. Inhibition of the Hippo pathway and activation of YAP1 can significantly improve liver regeneration.

3.make 3 columns dividing into gene name, function and link with usage1 in table form

Gene Name	Function	Link with USAG1
CTNNB1 (β-catenin)	Promotes hepatocyte proliferation and liver stem cell activation via Wnt signaling.	Inhibition of USAG1 could activate Wnt/β-catenin signaling, enhancing liver regeneration.
SHH (Sonic Hedgehog)	Stimulates liver tissue repair and progenitor cell expansion.	USAG1 inhibition may release suppression on the Hedgehog pathway, promoting liver regeneration.
YAP1	Regulates cell proliferation, survival, and liver regeneration through Hippo pathway inhibition.	USAG1 inhibition could activate YAP1 by disrupting the Hippo pathway, promoting liver cell growth.
CDKN1A (P21)	Cell cycle inhibitor that regulates hepatocyte proliferation.	Inhibiting USAG1 would likely downregulate CDKN1A, allowing hepatocyte division and regeneration.
LGR5	Stem cell marker and regulator of liver progenitor cell activation.	USAG1 inhibition could promote the activation of LGR5+ liver stem cells, aiding tissue regeneration.
HGF (Hepatocyte Growth Factor)	Stimulates hepatocyte proliferation and liver tissue repair.	USAG1 inhibition could increase HGF activity, enhancing liver regeneration and repair.
EGF (Epidermal Growth Factor)	Promotes cell growth and proliferation in liver regeneration.	USAG1 interference could upregulate EGF signaling, facilitating liver repair.
AXIN2	Negative regulator in Wnt/β-catenin signaling that controls β-catenin degradation.	USAG1 inhibition may reduce AXIN2 activity, stabilizing β-catenin and enhancing Wnt signaling for regeneration.
MYC	Drives cell cycle progression and proliferation, promoting tissue recovery.	Inhibition of USAG1 could activate MYC, leading to enhanced hepatocyte proliferation and liver regeneration.
NOTCH1	Regulates liver progenitor cell differentiation and maintains stem cell populations.	USAG1 inhibition could activate NOTCH1, promoting differentiation of progenitors into functional hepatocytes.

4.add 4th column to the previous table to include the stage that gene could interfere with liver regeneration process

Gene Name	Function	Link with USAG1	Stage in Liver Regeneration
CTNNB1 (β-catenin)	Promotes hepatocyte proliferation and liver stem cell activation via Wnt signaling.	Inhibition of USAG1 could activate Wnt/β-catenin signaling, enhancing liver regeneration.	Early to mid-phase; stimulates proliferation of hepatocytes and stem cells.
SHH (Sonic Hedgehog)	Stimulates liver tissue repair and progenitor cell expansion.	USAG1 inhibition may release suppression on the Hedgehog pathway, promoting liver regeneration.	Early-phase; activates liver progenitor cells and aids tissue repair.
YAP1	Regulates cell proliferation, survival, and liver regeneration through Hippo pathway inhibition.	USAG1 inhibition could activate YAP1 by disrupting the Hippo pathway, promoting liver cell growth.	Mid to late-phase; enhances cell survival and tissue repair.
CDKN1A (P21)	Cell cycle inhibitor that regulates hepatocyte proliferation.	Inhibiting USAG1 would likely downregulate CDKN1A, allowing hepatocyte division and regeneration.	Early-phase; facilitates hepatocyte entry into the cell cycle for proliferation.
LGR5	Stem cell marker and regulator of liver	USAG1 inhibition could promote the activation of	Early-phase; activates liver stem cells for
HGF (Hepatocyte Growth Factor)	Stimulates hepatocyte proliferation and liver tissue repair.	USAG1 inhibition could increase HGF activity, enhancing liver regeneration and repair.	Early to mid-phase; stimulates hepatocyte growth and tissue regeneration.
EGF (Epidermal Growth Factor)	Promotes cell growth and proliferation in liver regeneration.	USAG1 interference could upregulate EGF signaling, facilitating liver repair.	Early to mid-phase; enhances hepatocyte proliferation and wound healing.
AXIN2	Negative regulator in Wnt/β-catenin signaling that controls β-catenin degradation.	USAG1 inhibition may reduce AXIN2 activity, stabilizing β-catenin and enhancing Wnt signaling for regeneration.	Early-phase; stabilizes β-catenin, promoting proliferation and stem cell activation.
MYC	Drives cell cycle progression and proliferation, promoting tissue recovery.	Inhibition of USAG1 could activate MYC, leading to enhanced hepatocyte proliferation and liver regeneration.	Early to mid-phase; stimulates rapid hepatocyte division and tissue recovery.
NOTCH1	Regulates liver progenitor cell differentiation and maintains stem cell populations.	USAG1 inhibition could activate NOTCH1, promoting differentiation of progenitors into functional hepatocytes.	Mid to late-phase; supports differentiation of progenitor cells into mature hepatocytes.

5.predict stages of liver regeneration

1. **Injury and Initiation Phase (0-12 hours)**:
   In response to liver injury, inflammation and stress signals are rapidly activated. Growth factors like HGF and EGF are released to prepare the liver for regeneration.

2. **Proliferation Phase (12-48 hours)**:
   Hepatocytes and liver progenitor cells begin to proliferate, re-entering the cell cycle. Wnt/β-catenin and MYC signaling drive the rapid growth and division of liver cells.

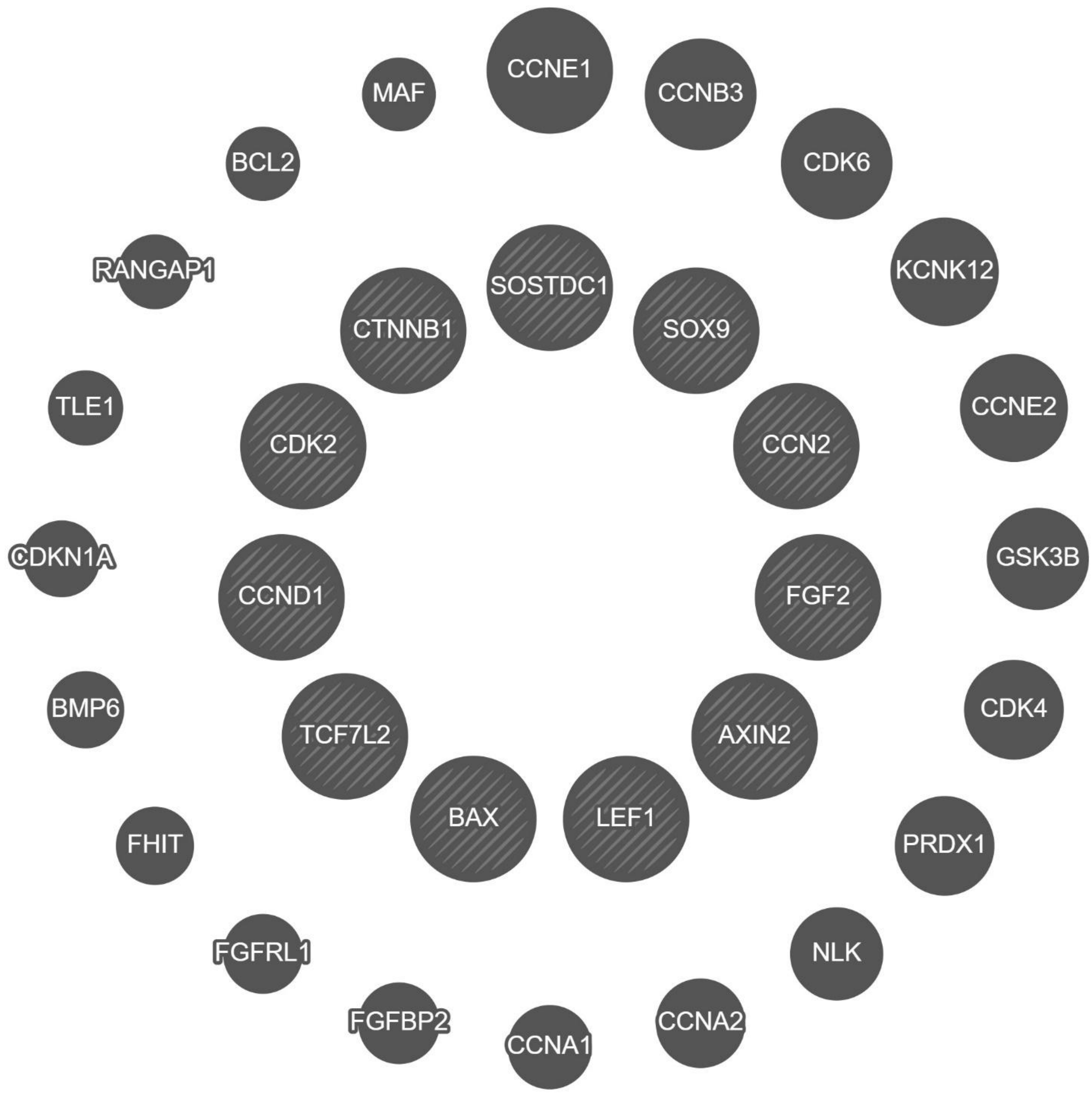
3. **Differentiation Phase (48-72 hours)**:
   Newly proliferated cells start differentiating into mature hepatocytes, restoring liver function. NOTCH1 and FOXO1 signaling help direct differentiation and support cell survival.

4. **Maturation and Recovery Phase (3-7 days)**:
   The liver undergoes tissue remodeling and recovery, with restored hepatocyte function and liver structure. Growth factors like HGF and EGF facilitate the final stages of liver tissue repair.

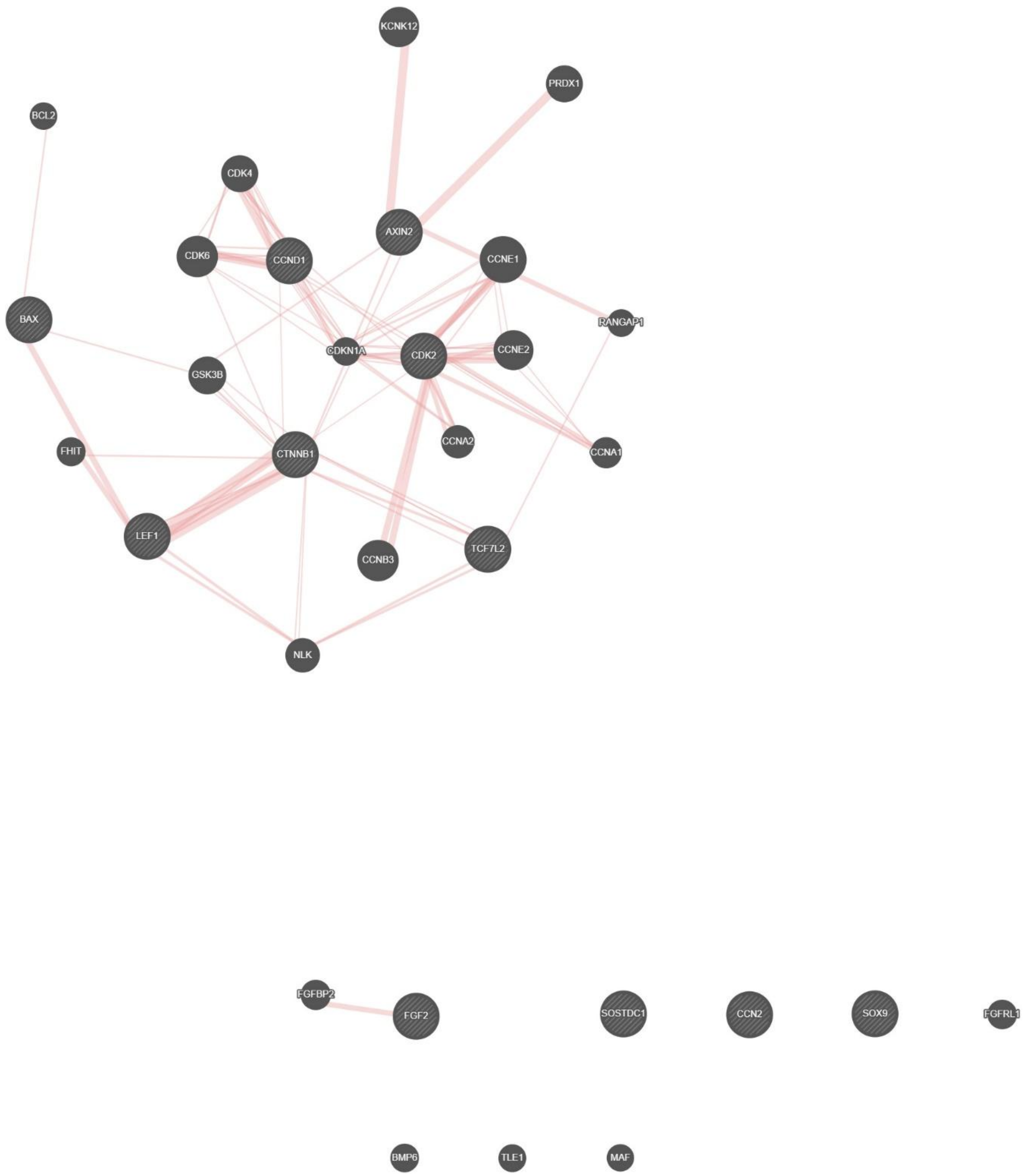
5. **Resolution Phase (7-14 days)**:
   Inflammation subsides, and liver regeneration completes, returning the liver to its normal size and function. The liver restores homeostasis, and any remaining damage resolves.

6. **Quiescence (After Recovery Phase)**:
   Once the liver has fully regenerated, it enters a quiescent state, maintaining normal function. The liver's growth signaling pathways return to baseline levels, ensuring stability.

Using GeneMANIA we add all suspected genes, and we got the following



According to GeneMANIA there is a physical interaction between the selected genes which means that there is molecular level interaction between them this interaction may be interaction between there encoded proteins



1. TCF7L2 (T-cell factor 7-like 2) –
   USAG1 may indirectly affect TCF7L2 by regulating Wnt/β-catenin signaling. USAG1 inhibits BMP signaling, which could modulate Wnt/β-catenin activity, thereby affecting the role of TCF7L2 in transcriptional regulation.

2. LEF1 (Lymphoid enhancer-binding factor 1) –
   USAG1 can influence the activity of LEF1 through Wnt/β-catenin signaling, as LEF1 binds with β-catenin to regulate gene expression, particularly in stem cell proliferation and differentiation. USAG1 inhibition could enhance this interaction by activating Wnt signaling.

3. CTNNB1 (β-catenin) –
   USAG1 regulates the Wnt/β-catenin pathway, and inhibiting USAG1 could stabilize CTNNB1, enhancing its interaction with LEF1 and TCF7L2 to promote gene transcription and liver regeneration.

4. AXIN2 –
   USAG1 may influence AXIN2 by modulating the Wnt/β-catenin pathway. AXIN2 acts as a negative regulator in this pathway, so USAG1 inhibition could disrupt the balance, favoring CTNNB1 activation.

5. CCND1 (Cyclin D1) –
   USAG1 may influence CCND1 expression by activating Wnt/β-catenin signaling. CCND1 promotes cell cycle progression, and its expression could be upregulated in response to CTNNB1 and LEF1 activation by USAG1 inhibition.

6. CCN2 (Connective tissue growth factor) –
   USAG1 might affect CCN2 expression through growth factor signaling pathways. While not directly interacting with USAG1, CCN2 is involved in tissue repair and could be influenced by HGF and FGF2, which are regulated by USAG1.

7. FGF2 (Fibroblast Growth Factor 2) –
USAG1 may interact with FGF2 through the regulation of growth factors. USAG1 inhibition could upregulate FGF2, which plays a crucial role in liver regeneration and tissue repair.

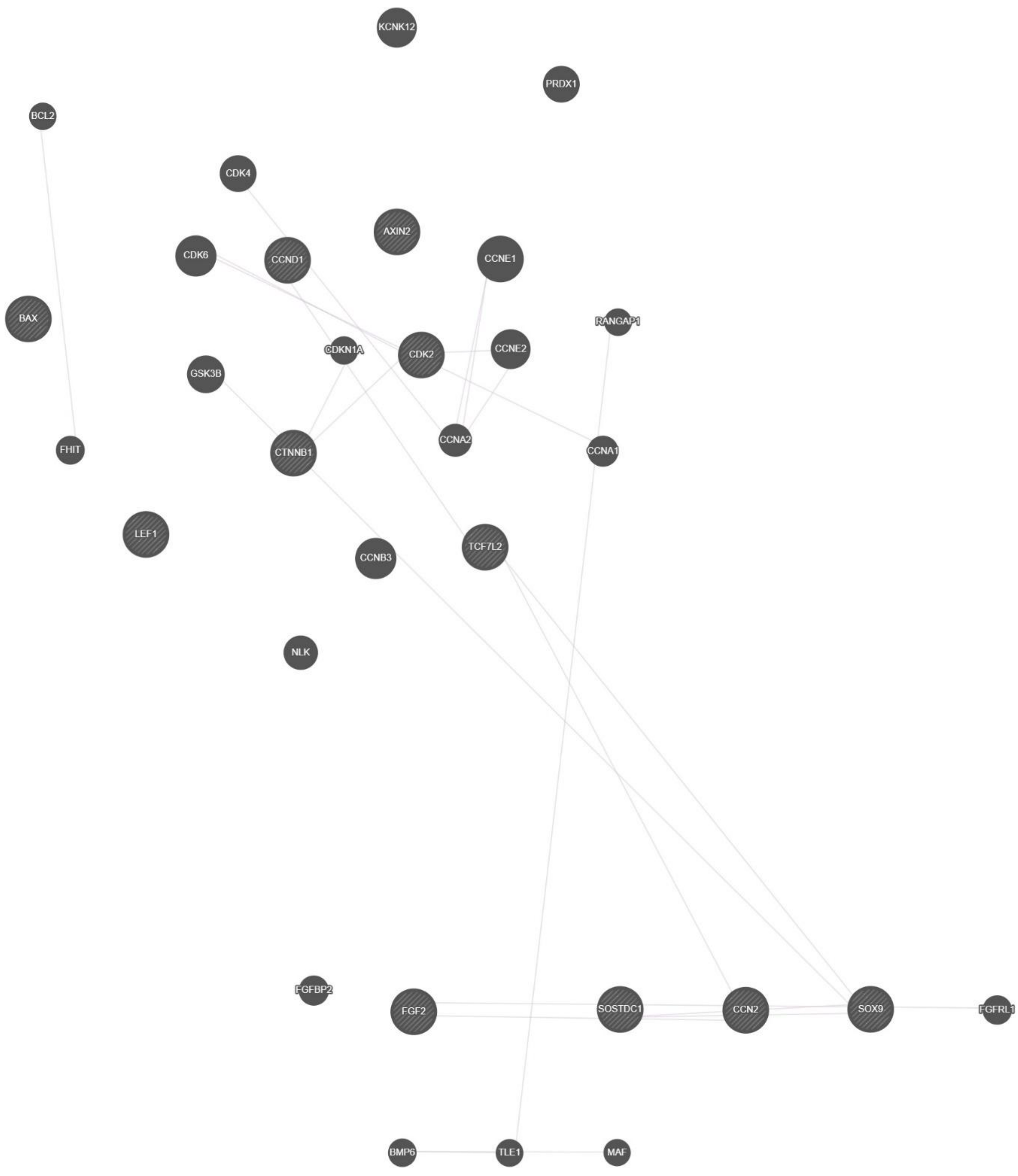
8. BAX (Bcl-2-associated X protein) –
USAG1 could affect BAX through regulation of apoptosis. USAG1 inhibition might promote hepatocyte survival by modulating apoptotic signals, potentially reducing BAX activity and increasing liver regeneration.

9. CDK2 (Cyclin-dependent kinase2)–
USAG1 could influence CDK2 activity by promoting cell cycle progression. The activation of the Wnt/β-catenin pathway might upregulate CCND1, leading to activation of CDK2 and cell division during liver regeneration.

10. HGF (Hepatocyte Growth Factor) –
USAG1 can regulate HGF expression, which plays a pivotal role in liver regeneration. USAG1 inhibition could enhance HGF signaling, promoting hepatocyte proliferation and survival.

11. SOX9 (SRY-box transcription factor 9) –
USAG1 might indirectly affect SOX9 by influencing pathways involved in liver progenitor cell activation. While not directly interacting, SOX9 plays a role in stem cell regulation, which could be influenced by USAG1 through Wnt and growth factor signaling.

Shared co-expression between two genes, according to GeneMANIA, means their expression levels are correlated in similar conditions or tissues. This suggests that the genes may be co-regulated and participate in related biological processes. Co-expression indicates



1. USAG1 and TCF7L2:
   - Co-expression may occur through the regulation of the Wnt/β-catenin pathway. Both genes are involved in transcriptional regulation, and USAG1 may modulate TCF7L2 activity via the Wnt signaling pathway.
2. USAG1 and LEF1:
   - LEF1 is a downstream target of Wnt/β-catenin signaling, and USAG1 inhibition may upregulate LEF1 expression, particularly in stem cell and regenerative contexts, resulting in co-expression during liver regeneration.
3. USAG1 and CTNNB1 (β-catenin):
   - USAG1 modulates the Wnt/β-catenin signaling pathway, so co-expression between USAG1 and CTNNB1 is expected, especially in tissues undergoing regeneration, like the liver.
4. USAG1 and AXIN2:
   - AXIN2 is involved in the regulation of Wnt/β-catenin signaling. Since USAG1 can affect this pathway, co-expression with AXIN2 might occur, though AXIN2 typically acts as a negative regulator of β-catenin signaling.
5. USAG1 and CCND1:
   - CCND1 (Cyclin D1) is involved in cell cycle regulation and is often upregulated by Wnt/β-catenin signaling. Therefore, USAG1 might co-express with CCND1 during cellular proliferation and regeneration.
6. USAG1 and CCN2:
   - CCN2 is involved in tissue repair, and USAG1 can modulate various growth factors like FGF2 and HGF, which may influence CCN2 expression. Co-expression might occur in the context of liver regeneration and fibrosis.

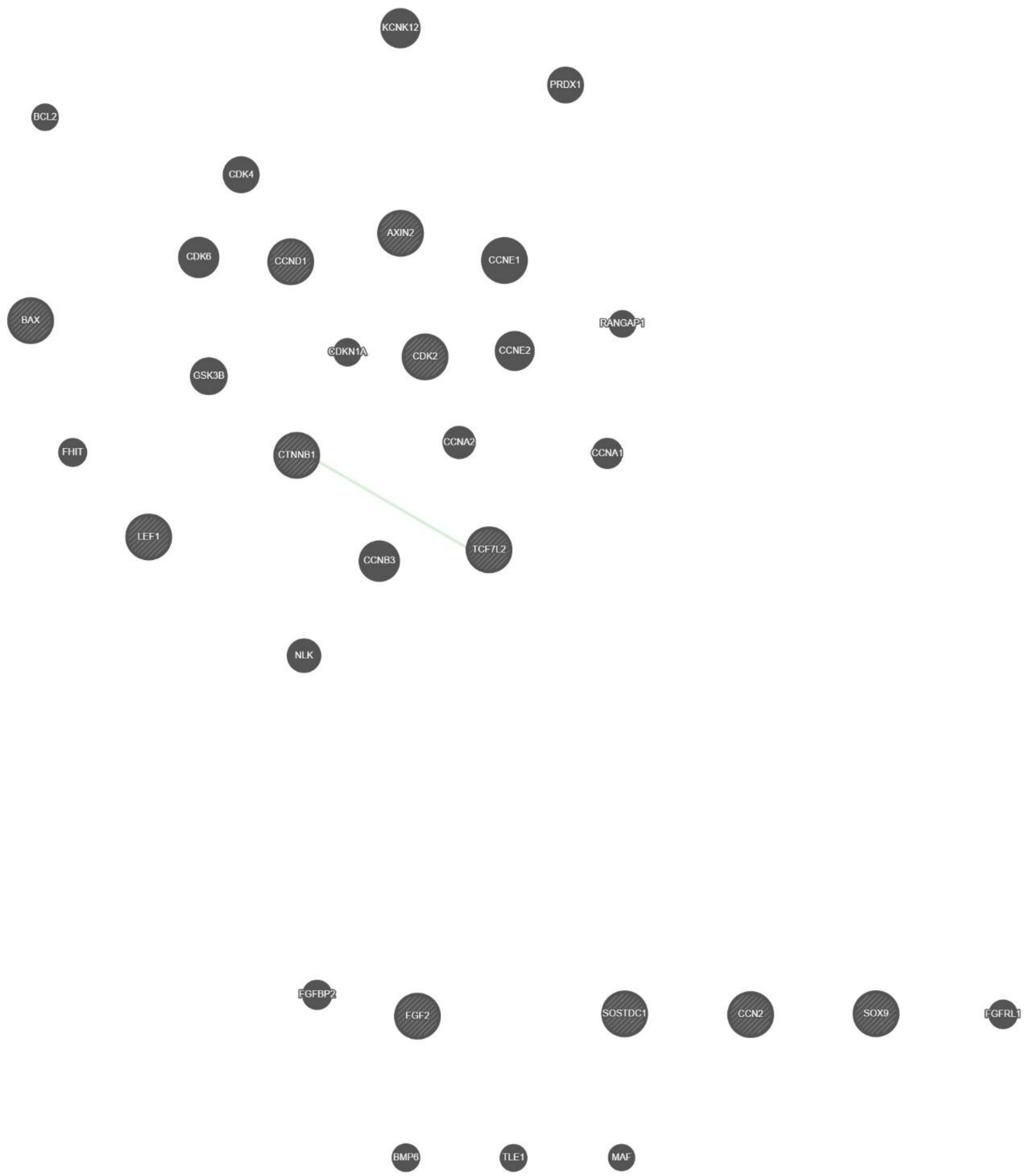
7. USAG1 and FGF2:
    - FGF2 plays a role in liver regeneration. USAG1 inhibition could enhance FGF2 expression, especially during tissue repair and regeneration, leading to co-expression in these processes.
8. USAG1 and BAX:
    - BAX regulates apoptosis, and while USAG1 typically promotes regeneration, there may be transient co-expression, particularly in the resolution phase of regeneration, where cell survival and death are balanced.
9. USAG1 and CDK2:
    - CDK2 regulates the cell cycle, and USAG1 could influence its expression through Wnt/β-catenin signaling and cell cycle control mechanisms, suggesting co-expression during liver cell proliferation.
10. USAG1 and HGF:

- HGF (Hepatocyte Growth Factor) is crucial for liver regeneration. USAG1 may regulate HGF expression by modulating other growth factors, leading to co-expression during liver repair and proliferation.

11. USAG1 and SOX9:

- SOX9 is involved in stem cell regulation, and its expression may be influenced by USAG1, particularly in liver progenitor cells. Co-expression could occur during liver regeneration and progenitor cell differentiation.

A genetic interaction between two genes, according to GeneMANIA, occurs when the genes influence each other's function or phenotype. This can involve mechanisms like epistasis, where one gene affects the expression of another, or synthetic lethality, where mutations in both genes cause a lethal phenotype. It may also involve regulatory feedback, where one gene modulates the expression of the other. These interactions highlight how genes collaborate within biological pathways and cellular processes.



1. USAG1 and TCF7L2:
   - USAG1 may genetically interact with TCF7L2, a transcription factor involved in the Wnt signaling pathway. USAG1 inhibits BMP signaling, which indirectly regulates Wnt activity. As a result, USAG1 might influence TCF7L2 activity in the context of regeneration by modulating Wnt signaling, which is crucial for cell proliferation and differentiation.
2. USAG1 and LEF1:
   - LEF1 functions downstream of Wnt/β-catenin signaling and interacts with β-catenin to regulate gene expression. USAG1 could genetically influence LEF1 through Wnt signaling, enhancing LEF1's ability to activate target genes involved in cell proliferation and differentiation, particularly during liver regeneration.
3. USAG1 and CTNNB1 (β-catenin):
   - USAG1 directly impacts the Wnt/β-catenin signaling pathway by inhibiting BMP signaling, leading to the stabilization and activation of CTNNB1 (β-catenin). This genetic interaction can result in increased transcription of Wnt/β-catenin target genes, promoting cell proliferation and tissue repair, such as during liver regeneration.
4. USAG1 and AXIN2:
   - AXIN2 is a negative regulator of the Wnt/β-catenin pathway, and USAG1 may genetically interact with AXIN2 to modulate this pathway. By inhibiting BMP signaling, USAG1 could reduce the expression of AXIN2, which in turn may enhance β-catenin signaling and promote liver regeneration.
5. USAG1 and CCND1:
   - CCND1 (Cyclin D1) is a key regulator of the cell cycle and is often upregulated by Wnt/β-catenin signaling. USAG1 could genetically enhance CCND1 expression by promoting Wnt/β-catenin activity, leading to increased cell proliferation during liver regeneration.

6. USAG1 and CCN2:

- CCN2 is involved in tissue repair and fibrosis. USAG1 could genetically interact with CCN2 through the regulation of growth factors like FGF2 and HGF. By modulating these factors, USAG1 may influence CCN2 expression during liver repair and fibrosis, promoting regeneration and tissue remodeling.

7. USAG1 and FGF2:

- FGF2 is a growth factor critical for liver regeneration and tissue repair. USAG1 may genetically interact with FGF2 by modulating upstream signaling pathways such as Wnt and BMP. This interaction could enhance the expression of FGF2, promoting hepatocyte proliferation and regeneration.

8. USAG1 and BAX:

- BAX is involved in the regulation of apoptosis. While USAG1 promotes liver regeneration, it could genetically interact with BAX by modulating apoptotic pathways. USAG1 may reduce BAX expression to promote cell survival during liver regeneration, preventing excessive apoptosis.

9. USAG1 and CDK2:

- CDK2 (Cyclin-dependent kinase 2) regulates cell cycle progression, particularly at the G1/S checkpoint. USAG1 could genetically interact with CDK2 through Wnt/β-catenin signaling and cell cycle regulation, promoting cell division and liver regeneration during tissue repair.

10. USAG1 and HGF:

- HGF (Hepatocyte Growth Factor) is a major regulator of liver regeneration. USAG1 could genetically interact with HGF by modulating growth factor signaling. By inhibiting BMP signaling, USAG1 might increase HGF expression, further promoting hepatocyte proliferation and liver repair.

11. USAG1 and SOX9:

SOX9 plays a role in stem cell regulation and liver progenitor cell activation. USAG1 may genetically interact with SOX9, particularly in the context of liver progenitor cell differentiation. USAG1 could influence SOX9 expression through modulation of Wnt signaling, which is crucial for stem cell maintenance and tissue regeneration.

Discussion:

1. Early Phase (First Few Hours):

USAG1

Upon liver injury, hepatocytes (liver cells) rapidly sense damage and begin to proliferate. Early signals include the release of growth factors and the activation of intracellular pathways like MAPK, Wnt, and JAK-STAT. If USAG1 is involved, it could modulate this early phase by regulating the signals that initiate cell proliferation Tcf7l2 During the early phase of liver regeneration, the liver cells (hepatocytes) respond to injury by initiating the early signaling cascades that help start tissue repair and proliferation.

TCF7L2

is involved in the activation of the Wnt/β-catenin signaling pathway, which is crucial for the initial response to liver damage. TCF7L2 acts as a transcriptional regulator that binds to β-catenin, and this interaction activates the expression of genes that control cell survival and initiate early cell division. This is especially important to kickstart the regeneration process. Additionally, TCF7L2 promotes the expansion of hepatocyte progenitor cells that can differentiate into hepatocytes to replace lost liver tissue.

LEF1

During the early phase of liver regeneration, LEF1 contributes to the initial response to liver injury. The liver rapidly begins repairing itself after injury, and LEF1 plays a crucial role in the activation of the Wnt/β-catenin signaling pathway, which is vital for initiating the regenerative process. In the early phase, LEF1 helped promote the survival of hepatocytes and progenitor cells in response to injury. This involves the activation of genes that promote cell proliferation and tissue repair. It also supports the activation of stem/progenitor cells in the liver, which can differentiate into mature hepatocytes to replace damaged or lost tissue.

CTNNB1

During the early phase of liver regeneration, after liver injury (such as partial hepatectomy or toxin-induced damage), CTNNB1 (β-catenin) is activated in response to the injury. β-catenin accumulates in the cytoplasm and translocate to the nucleus, where it interacts with T-cell factor/lymphoid enhancer-binding factor (TCF/LEF) transcription factors. This forms a complex that drives the expression of genes involved in cell survival and the initiation of regeneration. In the early phase, the role of CTNNB1 is to promote hepatocyte survival, prevent apoptosis, and initiate the proliferation of hepatocyte progenitor cells. It activates genes that are important for cell cycle progression and DNA repair, ensuring that the liver can respond quickly to the damage. β-catenin also contributes to the activation of liver progenitor cells, which can differentiate into hepatocytes and other liver cell types to replace lost tissue.

AXIN2

In the early phase of liver regeneration, following liver injury or partial hepatectomy, AXIN2 helps regulate the initiation of the regenerative process by influencing the Wnt/β catenin signaling pathway. AXIN2 acts as a negative regulator of Wnt signaling, and its expression can be modulated in response to liver damage. In this phase, the inhibition of Wnt signaling by AXIN2 is important to ensure that excessive activation of β-catenin does not occur prematurely, which could lead to abnormal proliferation. AXIN2 also plays a role in fine-tuning the activation of Wnt/β-catenin signaling in hepatocytes and progenitor cells, promoting the appropriate balance between cell proliferation and tissue repair. At this point, AXIN2 helps fine-tune the balance of Wnt signaling to avoid excessive or unregulated cell division while still allowing sufficient cellular responses to initiate the regeneration process.

CCND1

Cyclin D1 (encoded by CCND1) is activated in response to liver injury, such as partial hepatectomy or toxin-induced damage. During the early phase of liver regeneration, Cyclin D1 levels are upregulated as part of the initial response to liver damage. CCND1 plays a key role in initiating cell cycle entry for hepatocytes. As the liver responds to injury, Cyclin D1 promotes the transition of hepatocytes from G0 (quiescence) to G1 phase, where they are primed to enter the cell cycle and begin proliferation. The activation of CCND1 is critical for hepatocyte priming and early proliferation of progenitor cells. Cyclin D1 helps to break the quiescent state of hepatocytes, setting the stage for subsequent proliferation in response to the injury.

CCN2

In the early phase of liver regeneration, following liver injury or partial hepatectomy, CCN2 (CTGF) is upregulated as part of the response to tissue damage. CTGF is involved in wound healing and initial inflammation. During the early phase, it helps regulate the activation of hepatic stellate cells and fibroblasts, which are critical for ECM remodeling and repair. CTGF interacts with various growth factors, such as TGF-β, to promote fibroblast migration and the deposition of collagen and other ECM proteins, which is crucial for repairing the damaged tissue. Additionally, CTGF may stimulate hepatocyte survival by enhancing cell adhesion and promoting cellular signaling pathways that prevent apoptosis and support tissue integrity in the initial stages of injury recovery.

FGF7

In the early phase of liver regeneration, following liver injury such as partial hepatectomy or toxic damage, FGF7 is upregulated in response to liver damage. It helps initiate the regeneration process by stimulating the activation of hepatocytes and other liver cell types. FGF7 acts through its receptor FGFR2b, which is expressed on hepatocytes and non-parenchymal liver cells. During this phase, FGF7 helps activate hepatocytes and liver progenitor cells, promoting cell survival and proliferation. FGF7 also contributes to wound healing by influencing the activation of hepatic stellate cells and other fibroblasts, which are involved in the early phases of ECM remodeling. The early phase involves a pro-inflammatory response in which FGF7 helps manage cell responses to injury, promoting cellular survival, reducing apoptosis, and enhancing the repair process.

BAX

In the early phase of liver regeneration, following liver injury (such as partial hepatectomy or toxic damage), BAX is involved in regulating apoptosis in response to cellular stress and damage. During liver injury, BAX is activated as part of the intrinsic apoptotic pathway, which is triggered by various stress signals, including DNA damage, oxidative stress, or mitochondrial dysfunction. In this phase, BAX helps clear damaged cells by promoting apoptosis, preventing the proliferation of cells with damaged DNA or dysfunctional mitochondria. However, BAX activation needs to be carefully regulated to avoid excessive cell death. While apoptosis can promote tissue remodeling, an uncontrolled increase in BAX-induced apoptosis can impair liver regeneration. BAX expression levels are tightly controlled, as mild apoptosis can be beneficial in removing damaged hepatocytes, while excessive apoptosis could lead to impaired liver regeneration. This delicate balance helps create a reparative environment in the early phase.

CDK2

is initially inactive in the liver under normal conditions. However, following liver injury (such as partial hepatectomy or chemical damage), CDK2 activity is regulated through the upregulation of Cyclin E and Cyclin A, which activate CDK2 to drive hepatocyte re-entry into the cell cycle. In the early phase of liver regeneration, the liver is in a reactive state, and hepatocytes need to prepare for proliferation. CDK2 is activated as part of the initial cell cycle machinery to support the DNA replication process and the entry of hepatocytes into the S phase. During this phase, CDK2 interacts with Cyclin E, forming a complex that facilitates the G1 to S transition, promoting the DNA synthesis necessary for liver tissue recovery. At this stage, CDK2 helps activate the expression of genes required for DNA replication and cell cycle progression, ensuring that damaged liver cells are repaired or replaced by newly proliferated hepatocytes.

HGF

In the early phase of liver regeneration, following liver injury (such as partial hepatectomy, chemical damage, or toxic stress), the liver responds to the damage by increasing the secretion of HGF. HGF is produced mainly by non-parenchymal cells such as stellate cells, fibroblasts, and endothelial cells in response to tissue injury. These cells secrete HGF into the local environment where it interacts with hepatocytes to initiate the regeneration process. HGF binds to the c-MET receptor on hepatocytes, triggering intracellular signaling pathways that promote hepatocyte survival and activation. This is critical in preventing excessive apoptosis (cell death) and ensuring that the liver has enough viable cells to begin the regeneration process. HGF also plays a role in initiating hepatocyte proliferation, although this process is more pronounced in the proliferative phase. In the early phase, HGF helps initiate hepatocyte activation and prepares them for cell cycle entry.

SOX9

Its expression is low in normal, healthy hepatocytes but is upregulated in response to liver injury, particularly in liver progenitor cells (also known as oval cells) and other non-parenchymal cells. In the early phase, SOX9 is primarily involved in the activation of progenitor cells, which can differentiate into hepatocytes or biliary cells, depending on the cues in the microenvironment. In response to liver damage, SOX9 promotes stem-like behavior and the dedifferentiation of hepatocytes into a progenitor state. This helps repopulate the liver and initiates the process of tissue regeneration by facilitating hepatocyte reprogramming and progenitor cell expansion. SOX9 can cooperate with other transcription factors (such as HNF4α, FOXA2, and Notch signaling pathways) to induce a progenitor-like state in hepatocytes, preparing the liver for further regeneration and cell cycle re-entry.

2. Proliferative Phase (1-2 Days):

USAG1

During this phase, hepatocytes enter the cell cycle and undergo mitosis to replace lost tissue. USAG1 might influence this process by regulating the availability of critical growth factors or interfering with the extracellular matrix's ability to support cell division.

TCF7L2

In the proliferative phase, the liver undergoes rapid hepatocyte proliferation to restore liver mass. TCF7L2 continues to play a significant role by maintaining the activation of the Wnt/β-catenin signaling pathway during this period. The Wnt/β-catenin pathway, through TCF7L2, promotes hepatocyte proliferation, ensuring that the liver regenerates effectively. TCF7L2 is involved in maintaining hepatocyte self-renewal and preventing premature differentiation during the proliferative phase, allowing the liver to grow back to its original size. This is a critical phase where the liver needs to regenerate large numbers of hepatocytes to restore function.

LEF1

During the proliferative phase, LEF1 continues to play a crucial role by sustaining the Wnt/β-catenin pathway. This ensures that hepatocytes and progenitor cells proliferate rapidly to restore the liver’s mass. LEF1 acts as a coactivator of β-catenin, driving the expression of genes that are involved in hepatocyte proliferation and self-renewal. This allows the liver to regenerate the large numbers of hepatocytes needed to restore its structure and function. LEF1 also helps in maintaining the balance between proliferation and differentiation of liver cells, ensuring that the regeneration process occurs efficiently.

CTNNB1

During the proliferative phase, the liver undergoes rapid hepatocyte proliferation to restore its mass. The role of CTNNB1 (β-catenin) is central to hepatocyte proliferation. β-catenin activates several genes associated with cell division and proliferation, including those in the cyclin D1 and MYC family, which drive the cell cycle. This phase is characterized by compensatory hyperplasia, where the remaining hepatocytes proliferate to regenerate the liver mass. CTNNB1 ensures that hepatocytes divide efficiently without prematurely differentiating. β-catenin also maintains a balance between proliferation and differentiation, ensuring that hepatocytes proliferate rapidly enough to regenerate the liver but do not enter a differentiation state too soon.

AXIN2

During the proliferative phase, the liver undergoes rapid hepatocyte proliferation to restore liver mass. AXIN2 continues to modulate Wnt/β-catenin signaling to ensure that the proliferative response remains controlled and balanced. While Wnt/β-catenin signaling is required for hepatocyte proliferation, AXIN2 maintains a regulatory role in this process by preventing over-activation of β-catenin. This ensures that the liver's proliferative response does not lead to tumorigenesis or excessive growth. AXIN2 may also be involved in regulating the expression of specific genes that drive cell cycle progression and self-renewal of hepatocytes. Its role in this phase is crucial for maintaining controlled hepatocyte proliferation while preventing the uncontrolled cell division that could lead to abnormal liver growth. Additionally, AXIN2 can influence the differentiation of hepatocytes, ensuring that the newly proliferated cells mature properly during the regenerative process.

CCND1

In the proliferative phase, the liver undergoes rapid hepatocyte proliferation to restore its mass. Cyclin D1 plays a central role in driving the cell cycle during this phase. During this phase, Cyclin D1 is associated with CDK4 and CDK6, promoting the transition from the G1 phase to the S phase, allowing cells to replicate their DNA and divide. Cyclin D1 is crucial for hepatocyte proliferation, as it ensures that cells continue to progress through the cell cycle. Its expression peaks during the proliferative phase, allowing the liver to regenerate lost hepatocytes and restore tissue mass. Cyclin D1 also helps in the regulation of the cell cycle checkpoints, ensuring that the proliferative process occurs without errors, which is essential for the accurate regeneration of the liver. Importantly, Cyclin D1 ensures a balance between proliferation and differentiation, ensuring that hepatocytes continue to proliferate in the early stages without prematurely entering a differentiated state.

CCN2

During the proliferative phase, CTGF continues to play an essential role in supporting the proliferation of hepatocytes and liver progenitor cells. CCN2 (CTGF) works in conjunction with other growth factors, such as VEGF (vascular endothelial growth factor) and HGF (hepatocyte growth factor), to promote cell proliferation. It also modulates the activity of fibroblasts and hepatic stellate cells, contributing to vascular remodeling and enhancing the formation of new blood vessels in the regenerating liver. CTGF is involved in the ECM remodeling that is necessary for the proper structural integrity of the liver. During this phase, CCN2 helps in the deposition of new ECM components and assists in cell-cell signaling to maintain tissue architecture while allowing for the proliferation of hepatocytes. CTGF also regulates the balance between proliferation and differentiation of hepatocytes. By interacting with key signaling pathways, it ensures that hepatocytes proliferate sufficiently to regenerate the liver mass, but not excessively, thus maintaining the appropriate growth rate and preventing abnormal tissue development.

FGF7

During the proliferative phase, when hepatocytes and other liver cells are proliferating to restore liver mass, FGF7 plays a critical role in cell division and tissue regeneration. FGF7 stimulates hepatocyte proliferation by activating the FGFR2b receptor on hepatocytes, which leads to downstream signaling that promotes entry into the cell cycle, particularly the G1 to S phase transition. FGF7 is also involved in vascular remodeling, which is necessary for supplying oxygen and nutrients to regenerating liver tissue. It promotes angiogenesis (formation of new blood vessels) and hepatic sinusoid remodeling, ensuring that newly proliferated cells are adequately supplied. In this phase, FGF7 helps maintain the balance between proliferation and differentiation of hepatocytes, ensuring that the liver regenerates effectively without over proliferation or tumorigenesis.

BAX

During the proliferative phase, the liver undergoes intense hepatocyte proliferation to restore its mass and function. In this phase, BAX continues to play a critical role in regulating the survival of hepatocytes, but the focus shifts from apoptosis to cell survival in a controlled manner. As hepatocytes begin to proliferate and regenerate, BAX helps regulate the selection of healthy cells by promoting the death of cells with severe DNA damage or dysfunctional cellular machinery, thus preventing malignant transformation or the proliferation of cells with compromised integrity. BAX also plays a role in mitochondrial integrity. By promoting apoptosis in damaged or dysfunctional hepatocytes, BAX ensures that only healthy hepatocytes proceed through the cell cycle and contribute to tissue regeneration. BAX helps maintain a balance between apoptosis and proliferation. Although the proliferative phase is characterized by hepatocyte division, BAX ensures that only viable cells are allowed to proliferate, preventing potential risks associated with uncontrolled growth of damaged cells. The role of BAX in this phase may also involve the regulation of the cell cycle and supporting the integrity of the liver tissue during regeneration by eliminating unwanted cells.

CDK2

The proliferative phase is characterized by rapid hepatocyte division to restore liver mass. CDK2 plays a central role in this phase by regulating the G1 to S phase transition of the cell cycle and facilitating DNA replication and cell division. In this phase, CDK2 becomes activated through its association with Cyclin E and Cyclin A. These complexes promote the phosphorylation of key targets involved in cell cycle progression, allowing hepatocytes to enter and progress through the S phase where DNA replication occurs. CDK2 activity supports hepatocyte proliferation by ensuring timely cell cycle progression and maintaining the integrity of the regenerating tissue. It is critical for the efficient and controlled division of hepatocytes, which is necessary to restore liver function and mass following injury. The proliferative phase also involves the elimination of damaged or senescent cells, and CDK2's role in regulating the cell cycle checkpoints ensures that cells with damaged DNA do not proliferate. The activation of CDK2 needs to be tightly controlled to prevent uncontrolled cell division that could lead to tumorigenesis.

HGF

The proliferative phase is marked by the rapid division of hepatocytes to restore liver mass and function. HGF is a key player in this phase, promoting hepatocyte proliferation and mitogenesis. After the liver injury, HGF binds to c-MET receptors on hepatocytes, activating several intracellular signaling pathways, such as the PI3K-Akt pathway and the MAPK pathway, which regulate cell survival, proliferation, and migration. This signaling cascade helps stimulate DNA replication and the entry of hepatocytes into the S phase of the cell cycle. HGF is crucial for re-initiating hepatocyte division and mass restoration. During this phase, HGF signaling not only promotes the proliferation of hepatocytes but also aids in their migration, facilitating the reconstruction of liver architecture after damage. The proliferative phase can last several days to weeks, depending on the extent of injury. HGF signaling plays a central role in the expansion of hepatocyte populations required to regenerate the liver. HGF also enhances the epithelial-mesenchymal transition (EMT) process, enabling hepatocytes to transition between different functional states during liver tissue remodeling.

SOX9

During the proliferative phase, SOX9 continues to influence the proliferation of liver progenitor cells. These cells are important for liver regeneration, especially in situations where hepatocyte proliferation is insufficient or there is significant liver damage. SOX9 is involved in maintaining the stem/progenitor cell pool, promoting the expansion of these cells and directing their differentiation into hepatocytes and biliary cells as needed. It regulates liver progenitor cell proliferation by interacting with other signaling pathways (e.g., Wnt/β-catenin, Notch, and FGF signaling) and contributing to the local environment that supports regeneration. In this phase, SOX9 can regulate the cell cycle of progenitor cells and influence their differentiation pathways. This ensures that the liver can produce sufficient numbers of hepatocytes or biliary cells, depending on the type of injury. SOX9 also helps prevent premature differentiation of progenitor cells, ensuring that these cells remain in an undifferentiated state long enough to expand the progenitor pool before differentiation begins.

3. Remodeling Phase (Weeks):

USAG1

Liver regeneration also involves tissue remodeling and re-establishing functional architecture. USAG1 could play a role in regulating how the regenerated tissue integrates into the pre-existing liver structure.

TCF7L2

During the remodeling phase, the liver undergoes fine-tuning to restore its normal structure. This involves changes in liver architecture, including the regeneration of blood vessels and the reorganization of the extracellular matrix (ECM). TCF7L2 may continue to regulate Wnt signaling, which is involved in cellular differentiation and the maturation of hepatocytes. This ensures that newly regenerated hepatocytes acquire proper function and that the liver's architecture is reorganized appropriately. TCF7L2 could also be involved in the regulation of genes that control the balance between proliferation and differentiation, helping the liver to transition from a proliferative state to a more differentiated and functional state.

LEF1

During the remodeling phase, the liver begins to reorganize its structure and architecture. This phase involves the remodeling of the extracellular matrix (ECM) and the maturation of newly regenerated hepatocytes into fully functional cells. LEF1, through its role in Wnt/β-catenin signaling, helps regulate the differentiation of hepatocytes. This ensures that hepatocytes mature properly and integrate into the liver architecture. LEF1 also helps modulate the transition from a proliferative state to a more differentiated state. It ensures that newly regenerated hepatocytes stop proliferating and start differentiating into fully functional liver cells, which is crucial for the restoration of normal liver function.

CTNNB1

The remodeling phase focuses on the reorganization of the liver tissue, including the regeneration of the extracellular matrix (ECM) and the proper alignment of hepatocytes within the liver architecture. CTNNB1 (β-catenin) remains involved in tissue remodeling, though its role now shifts more toward regulating differentiation and the maturation of newly generated hepatocytes. β-catenin continues to drive the maturation and functional differentiation of hepatocytes, ensuring that newly proliferated cells acquire proper functions, such as bile production and metabolic activity. β-catenin also contributes to the stabilization of the liver's architecture, promoting the organization of hepatocytes into functional lobules and the integration of regenerating t issue into the existing liver structure.

AXIN2

The remodeling phase is characterized by the restructuring of the liver, including the organization of hepatocytes and the remodeling of the extracellular matrix (ECM). In this phase, the newly proliferated hepatocytes differentiate and integrate into the existing liver architecture. AXIN2 continues to regulate the Wnt/β-catenin pathway, but now its role shifts towards controlling the differentiation of hepatocytes and ensuring proper tissue organization. The liver needs to transition from a highly proliferative state to a more differentiated, functional state. AXIN2 helps regulate the balance between proliferation and differentiation of hepatocytes. It ensures that while the liver continues to regenerate, cells do not remain in an undifferentiated proliferative state but instead mature and integrate into the liver's normal tissue structure. Additionally, AXIN2 may be involved in the remodeling of the ECM, a key component of the tissue architecture, to facilitate proper tissue regeneration and restoration of normal liver function.

CCND1

During the remodeling phase, the liver transitions from a highly proliferative state to a more differentiated and mature state. While the focus shifts from proliferation to tissue organization and ECM remodeling, Cyclin D1 continues to play a role in regulating cell cycle progression, although its expression typically decreases compared to the proliferative phase. Cyclin D1 is involved in ensuring the proper differentiation of hepatocytes. While the liver still undergoes some cell cycle progression, Cyclin D1 helps guide the liver cells to exit the cell cycle at the appropriate time to achieve full differentiation and functional maturity. Cyclin D1 may also support the reorganization of liver architecture, as hepatocytes mature and integrate into the restored tissue. However, its expression is typically reduced as the liver moves towards a more stable, differentiated state. The remodeling phase is characterized by hepatocytes becoming fully functional, and Cyclin D1 helps ensure that this transition occurs without excessive cell cycle activity or unwanted proliferation.

CCN2

The remodeling phase focuses on tissue reorganization, ECM remodeling, and functional restoration of the liver architecture. CTGF continues to play a key role in this process. During the remodeling phase, CCN2 (CTGF) facilitates the maturation and differentiation of hepatocytes, guiding them to adopt their mature, functional state. It helps regulate the transition from proliferation to differentiation, ensuring that hepatocytes become fully functional and contribute to the restored liver architecture. CTGF modulates the remodeling of the ECM, which is crucial for the reorganization of the t issue structure. It supports the recovery of liver sinusoids, restoration of vascular networks, and the proper arrangement of hepatocytes into functional lobules. In this phase, CCN2 helps prevent fibrosis by controlling hepatic stellate cells and modulating TGF-β signaling. If unchecked, fibrosis could lead to liver dysfunction, so CTGF ensures that the liver heals without excessive scar tissue formation.

FGF7

The remodeling phase is characterized by reorganization of the liver tissue, including the maturation of newly proliferated hepatocytes and the restoration of the liver’s architecture. During this phase, FGF7 continues to play an important role in supporting t issue repair and ensuring proper cell differentiation. FGF7 promotes ECM remodeling by influencing hepatic stellate cells and fibroblasts, which contribute to the restructuring of the liver's extracellular matrix (ECM). The balance of ECM deposition and degradation is critical to restore the liver’s structure and prevent excessive scarring or fibrosis. Additionally, FGF7 contributes to the restoration of liver sinusoidal architecture and vascular networks as the liver tissue matures. The angiogenic effects of FGF7 continue to support the development of a fully functional vasculature, which is essential for liver function and regeneration. This phase marks a transition from rapid proliferation to maturation and differentiation of hepatocytes, with FGF7 promoting the final stages of hepatocyte differentiation and functional recovery.

BAX

During the remodeling phase, the liver transitions from rapid proliferation to more controlled tissue reorganization and maturation. BAX remains important in regulating the cell death program in response to changes in the cellular and tissue architecture. BAX helps ensure the proper differentiation of hepatocytes, as excessive apoptosis during this phase can disrupt liver architecture and lead to pathological tissue remodeling. BAX contributes to the removal of senescent or damaged cells that could otherwise hinder proper liver tissue architecture and function. For example, it can promote the death of cells that failed to complete differentiation or those that are irreversibly damaged. Additionally, BAX may help regulate the transition of hepatocytes from a highly proliferative state to a more differentiated, functional state. Its activity helps maintain a balance between proliferation and differentiation, ensuring that only appropriate, mature hepatocytes continue to participate in liver function.

CDK2

The remodeling phase of liver regeneration involves the maturation and reorganization of the liver tissue, with hepatocytes transitioning from a proliferative state to a more differentiated, functional state. CDK2 continues to play a role in maintaining cell cycle progression, but its activity is now more focused on differentiation and the final steps of liver tissue reorganization. In this phase, hepatocytes may exit the cell cycle and enter a quiescent state as they mature. The expression of Cyclin A, which is complex with CDK2, may shift toward a role in regulating cell cycle exit and differentiation. CDK2 is involved in regulating the expression of genes required for the maturation of hepatocytes and proper tissue remodeling. CDK2 also plays a role in maintaining cellular integrity by ensuring that hepatocytes differentiate properly and contribute to the organization of the liver's architecture. Disruption of CDK2 activity at this stage could impair tissue organization and contribute to fibrosis or incomplete regeneration.

HGF

In the remodeling phase, liver tissue begins to reorganize, and hepatocytes transition from a highly proliferative state to more mature, differentiated cells. Here, HGF continues to play an essential role in tissue repair, but its function shifts towards tissue reorganization and maturation. During this phase, HGF helps in the remodeling of the extracellular matrix (ECM) and promotes the recovery of normal liver architecture. This is critical for regenerating not only the hepatocytes but also the surrounding support structures, such as vascular networks and fibroblasts. While HGF continues to promote hepatocyte survival, its role in proliferation decreases compared to the earlier stages. The focus during this phase is on cell differentiation, maturation, and re-establishing liver functions. HGF also aids in the restoration of tissue integrity by facilitating cell migration, especially in areas of liver damage where cells need to move to restore tissue continuity.

SOX9

In the remodeling phase, the liver begins to re-establish its structural and functional integrity. During this phase, SOX9 plays a role in tissue organization and in ensuring that the liver architecture is restored after injury. SOX9 supports the differentiation of liver progenitors into mature hepatocytes or biliary epithelial cells, depending on the specific needs of the tissue at the time. It helps control the epithelial-to-mesenchymal transition (EMT) process, which is important for the proper differentiation and organization of hepatocytes and bile duct cells. SOX9 can also contribute to the remodeling of the extracellular matrix (ECM) and the restoration of vascular networks, which are necessary for proper liver function and regeneration. The remodeling phase also involves the fine-tuning of liver cell populations, where SOX9 helps to balance progenitor cell differentiation with the regeneration of mature hepatocytes, ensuring that the liver tissue is properly organized and functionally restored.

4. Resolution and Maintenance (1-2 Months):

USAG1

Once regeneration is complete, there needs to be a balance between new tissue formation and the maintenance of liver function. USAG1 could potentially modulate this balance, helping to ensure that the liver does not over-regenerate or lose its functional integrity.

TCF7L2

In the resolution and maintenance phase, the liver transitions to a stable state where regeneration slows down, and the tissue matures and maintains homeostasis. TCF7L2 continues to regulate Wnt/β-catenin signaling, but now it is likely shifting toward maintaining liver homeostasis. Its role here involves ensuring that the regenerated liver maintains its function and that hepatocytes are maintained in a differentiated state. At this stage, TCF7L2 may also help with the repair of damaged liver structures, ensuring that fibrosis or scarring does not persist, and the liver returns to a functional and stable state.

LEF1

In the resolution and maintenance phase, LEF1 helps in the long-term stability of the liver after regeneration. This phase involves the stabilization of liver architecture and maintenance of liver function. LEF1, through its regulation of Wnt/β-catenin signaling, supports the maintenance of liver homeostasis. It helps ensure that the liver's cellular composition remains stable and that f ibrosis or scarring does not occur, promoting a return to normal liver function. LEF1 also contributes to tissue remodeling and repair by maintaining the appropriate balance between cell proliferation and differentiation. It is involved in ensuring that the liver maintains its regenerative capacity, but without excessive cell growth, which could lead to liver damage or cancer.

CTNNB1

In the resolution and maintenance phase, the regeneration process slows down as the liver reaches its full functional capacity. The role of CTNNB1 (β-catenin) is to maintain liver homeostasis and ensure that regeneration stops when the liver has sufficiently recovered. β-catenin maintains liver function by supporting hepatocyte survival and preventing excessive fibrosis or scarring. It plays a role in the final fine-tuning of hepatocyte differentiation, ensuring that liver cells maintain their specialized functions in the mature liver. β-catenin also plays a role in the maintenance of liver architecture and the prevention of abnormal growth or tumorigenesis. Once regeneration is complete, CTNNB1 helps to ensure that the liver does not over-proliferate and that normal tissue homeostasis is preserved.

AXIN2

In the resolution and maintenance phase, the liver has largely regenerated, and the focus shifts to maintaining homeostasis and preventing excessive fibrosis or scarring. AXIN2 plays a role in fine-tuning the levels of Wnt/β-catenin signaling to ensure that the regeneration process does not continue indefinitely and that the liver does not enter a state of chronic overgrowth or fibrosis. AXIN2 helps prevent excessive cell proliferation and supports the stabilization of the regenerated liver tissue. It ensures that the liver maintains functional homeostasis and prevents the development of abnormal tissue, including fibrosis or tumorigenesis. AXIN2 also likely participates in maintaining the differentiated state of hepatocytes and supporting their long-term function in the liver.

CCND1

In the resolution and maintenance phase, liver regeneration slows down, and the focus shifts to maintaining liver homeostasis and stabilizing the liver's architecture. Cyclin D1 continues to play a role in maintaining hepatocyte homeostasis but at lower levels than in the proliferative phase. It helps regulate cell cycle exit and the maintenance of a non-proliferative state in mature hepatocytes. During this phase, Cyclin D1 is involved in regulating tissue stability, preventing excessive cell division and ensuring that the liver reaches its final functional state. This is crucial to prevent the liver from entering a state of tumorigenesis or excessive growth. Cyclin D1's role in the resolution phase is to help maintain a proper balance between cell cycle arrest and functional maintenance, ensuring that the liver's regenerative capacity does not become deregulated after the tissue is repaired.

CCN2

In the resolution and maintenance phase, CTGF plays a role in maintaining liver homeostasis and stabilizing the ECM. By this phase, the liver has largely restored its mass and function. CCN2 (CTGF) helps maintain the balance between ECM deposition and remodeling to ensure long-term structural integrity of the liver. It helps regulate the stabilization of newly synthesized ECM components, preventing excessive fibrosis or scarring while maintaining the liver's ability to function properly. CTGF also contributes to the prevention of excessive fibrosis, ensuring that the ECM does not accumulate to levels that would impede normal liver function. In this phase, CTGF supports the long-term functional maintenance of hepatocytes and liver vasculature, ensuring that the liver remains in a state of homeostasis, free from overgrowth or uncontrolled remodeling.

FGF7

In the resolution and maintenance phase, the liver reaches its final, stable structure and function. FGF7 helps maintain tissue integrity and homeostasis, ensuring that hepatocytes remain in a differentiated, functional state. Although the liver regeneration process slows down, FGF7 continues to support the stabilization of the ECM, preventing excessive fibrosis and ensuring that the liver architecture remains intact. FGF7 also contributes to maintaining vascular integrity, ensuring that the newly formed blood vessels remain functional and continue to support liver tissue. In the maintenance phase, FGF7 helps maintain cell survival and functional differentiation, ensuring that the liver does not undergo excessive regeneration or fibrosis and that it continues to perform its metabolic functions properly.

BAX

In the resolution and maintenance phase, the liver is mostly restored, and the focus shifts to maintaining homeostasis and functional integrity. Here, BAX continues to play a role in maintaining tissue integrity by promoting the removal of any residual damaged or dysfunctional cells that could disrupt liver homeostasis. BAX ensures that apoptosis is tightly regulated during this phase, preventing the accumulation of damaged or aged cells while preventing excessive cell death that could hinder tissue stability and function. The resolution phase requires careful regulation of cell survival, as BAX-induced apoptosis can lead to excessive tissue damage if overactivated. In this context, BAX ensures that the regenerating liver maintains the proper balance of cell turnover and cell survival while avoiding fibrosis or excessive cell loss. The maintenance of a functional, non-proliferative state is essential in this phase, and BAX helps regulate the exit from the cell cycle for hepatocytes that have already completed their regeneration, ensuring they do not re-enter an unwanted proliferative state.

CDK2

In the resolution and maintenance phase, liver regeneration slows down as the organ returns to a more stable, homeostatic state. CDK2 plays a reduced but still important role in ensuring that hepatocytes are maintained in a differentiated and quiescent state. At this stage, CDK2 activity decreases as hepatocytes exit the cell cycle and enter a state of quiescence or differentiation. Cyclin E and Cyclin A levels drop, and CDK2 activity is downregulated, promoting the return to a stable, non-proliferative state. CDK2 may still be involved in the maintenance of tissue integrity and the periodic renewal of liver cells, but its role in active proliferation is diminished. The liver's structural and functional integrity is now maintained by quiescent hepatocytes and non-parenchymal cells like hepatic stellate cells and Kupffer cells. The resolution phase focuses on resolving any remaining damage, reducing inflammation, and maintaining the balance between cell survival and tissue homeostasis. CDK2 ensures that this balance is preserved by preventing unwanted cell cycle re-entry and ensuring the maintenance of liver tissue structure.

HGF

In the resolution and maintenance phase, liver regeneration slows as the organ returns to its normal structure and function. Here, the role of HGF shifts towards ensuring homeostasis and long-term maintenance of the regenerated liver. HGF continues to support hepatocyte maintenance, ensuring that cells remain in a quiescent, non-proliferative state, but still capable of responding to injury if it occurs again. It helps maintain the functional integrity of the liver by supporting cell survival and tissue repair in response to minor damage. HGF signaling during this phase ensures that hepatocytes are preserved in a functional, mature state and that the liver remains capable of performing essential metabolic and detoxification functions. HGF also plays a role in modulating the fibrotic response. By controlling fibrosis and aiding the remodeling of the extracellular matrix, HGF ensures that the liver does not develop excessive scar tissue that could impair its function in the long term.

SOX9

In the resolution and maintenance phase, the liver regeneration process begins to slow down, and the liver stabilizes back to homeostasis. SOX9's role in this phase becomes more focused on tissue maintenance and preventing fibrosis. As the liver reaches its full regenerative capacity, SOX9 helps to maintain the differentiated state of the newly generated hepatocytes, ensuring that these cells do not revert to a progenitor-like state and disrupt the liver's function. SOX9 also plays a role in preventing excessive fibrosis by balancing the activities of fibroblasts and stellate cells, ensuring that scarring is minimized during the regenerative process. This is particularly important because excessive ECM deposition and fibrosis can impair liver function and regeneration. During this phase, SOX9 helps ensure liver homeostasis by regulating the turnover of liver progenitor cells and hepatocytes, contributing to long-term tissue maintenance and stability.

AI predicted that the liver regeneration process will be divided into 6 stages

1.injury and initiation phase

2.proliferation phase

3.differentiation phase

4.maturation phase

5.resolustion phase

6.quiescence phase

Using 10 genes -CTNNB1 -YAP1 -CDKN1A -LGRS -HGF -EGF -AXIN2 -MYC -NOTCH1

We excluded 6 genes as they are -SHH/YAP1: no expression in liver during critical phases and rule similarity to SOX9 and

better expression to SOX9.

For the following reason

-efficient expression in liver in parallel to other critical genes that serve the ultimate goal

This process happens during 4 stages

1. Early Phase (First 24–48 hours)

The early phase of liver regeneration begins immediately after liver injury (e.g., partial

hepatectomy, toxic damage, or viral infection). The major events in this phase focus on the activation of hepatocytes and progenitor cells.

Cellular Response: In the early phase, there is initial liver injury or hepatocyte death that triggers a cascade of regenerative signals.

Signaling Pathways: Key molecular signaling pathways are activated, such as:

Hepatocyte growth factor (HGF): HGF is released in response to injury and activates c-Met receptors, leading to hepatocyte proliferation and liver regeneration.

Notch signaling: Promotes stem cell activation and differentiation into hepatocytes and biliary cells.

Inflammatory Cytokines: TNF-α and IL-6 are elevated and promote cell survival and proliferation of hepatocytes.

Wnt/β-catenin signaling: Important for stem cell activation, proliferation, and self-renewal of liver progenitors.

Injury Response: Liver progenitor cells (also known as oval cells) are activated and proliferate. In response to severe injury, progenitor cells help in the regeneration of both hepatocytes and biliary cells.

2. Proliferative Phase (2–4 Weeks)

The proliferative phase is characterized by rapid expansion and proliferation of hepatocytes and progenitor cells to restore liver mass and function. This phase involves tissue-specific regeneration.

Cellular Proliferation:

Hepatocytes begin to re-enter the cell cycle, especially quiescent hepatocytes (G0 phase), which rapidly proliferate to restore the liver mass.

Progenitor cells (which may have been activated in the early phase) proliferate and differentiate into hepatocytes and biliary epithelial cells.

Signaling Pathways:

HGF continues to drive hepatocyte proliferation.

Growth factors, such as FGFs (fibroblast growth factors), EGF (epidermal growth factor), and TGF-α (transforming growth factor-alpha), promote liver cell proliferation.

Wnt/β-catenin signaling maintains progenitor cell proliferation and hepatocyte regeneration.

Notch signaling also regulates stem cell maintenance, ensuring that liver progenitors are replenished without prematurely differentiating.

Angiogenesis: Blood vessel formation is activated to supply nutrients and oxygen to regenerating tissue, ensuring adequate blood flow and support.

3. Remodeling Phase (3–6 Weeks)

The liver begins to regain its normal architecture, and the regeneration process shifts from cell proliferation to tissue maturation.

Resolution of Inflammation: Inflammatory responses that were activated in the early phase start to resolve, and immune cells such as macrophages clear any remaining debris or damaged tissue.

Tissue Maturation:

Hepatocytes mature, and the liver structure starts returning to its normal architecture.

Matrix remodeling occurs as extracellular matrix proteins are degraded and replaced by new tissue.

The liver vasculature undergoes remodeling to restore proper blood flow.

Fibrosis Regulation: If the injury was severe, there might be a fibrotic response. Hepatic Stellate cells become activated and produce collagen, leading to fibrosis. However, in the remodeling phase, the liver works to resolve fibrosis to restore normal tissue function.

4. Resolution and Maintenance Phase (6 Weeks and Beyond)

The resolution and maintenance phase represents the final stage of liver regeneration, where the liver achieves its full regenerative capacity and restores its function and homeostasis.

Liver Homeostasis: The liver now maintains normal function and tissue architecture. This phase ensures that the liver's stem/progenitor cell pool is maintained, and hepatocytes are performing their normal metabolic,detoxification, and storage functions.

Maintenance of Regenerated Liver Mass: After the initial phase of regeneration, the liver now focuses on preserving the new liver mass and maintaining its structure over the long term. The liver retains the capacity for self-renewal and tissue repair if needed in the future.

Immune Regulation: Any chronic inflammation or immune response is resolved, with a return to immune tolerance in the liver. This prevents any ongoing immune-mediated damage.

Fibrosis Resolution: If fibrosis occurred during the proliferative or remodeling phases, the liver works to resolve the fibrosis, and the scar tissue is replaced with normal liver tissue.

Stem Cell Quiescence: Once the liver regenerates to its original size, liver progenitor cells may return to a quiescent state, remaining in dormancy unless future injury activates them again.

Conclusion:

Liver Regeneration as a Model: The project aims to leverage liver regeneration as a model for understanding tooth regeneration, focusing on the role of gene therapy in modulating key processes.

Gene Therapy's Role: Gene therapy offers the potential to precisely target and manipulate genes involved in tissue regeneration, providing a powerful tool for therapeutic intervention.

USAG1 Inhibition: Inhibiting USAG1, a negative regulator of regeneration, is hypothesized to activate key pathways involved in liver regeneration, such as Wnt/β-catenin signaling.

Key Genes in Liver Regeneration: Several genes are crucial for liver regeneration, including:

Wnt/β-catenin pathway genes: TCF7L2, LEF1, CTNNB1, AXIN2

Cell cycle regulators: CCND1, CDK2

Growth factors: HGF, FGF2

Apoptotic regulators: BAX

Other important genes: CCN2, SOX9

Regeneration Stages:

Liver regeneration involves distinct stages: injury and initiation, proliferation, differentiation, maturation and recovery, resolution, and quiescence.

Gene-Gene Interactions: GeneMANIA analysis revealed potential physical, genetic, and co-expression interactions among the identified genes, suggesting complex interplay in regulating liver regeneration.

Overall, the project aims to:

Advance understanding of regenerative mechanisms: By studying liver regeneration, researchers hope to gain insights into fundamental processes that can be applied to other tissues, including teeth.

Develop novel gene therapies:

The research seeks to establish a proof-of-concept approach for using gene therapy to induce liver regeneration, with the long-term goal of translating these findings into clinically viable therapies for various regenerative medicine applications.

Further Research Directions:

In-depth experimental studies are needed to validate the predicted gene interactions and their roles in liver regeneration.

Preclinical Models: Animal models can be used to test the efficacy and safety of gene therapy approaches targeting the identified genes.

Translational Research: Ultimately, the findings need to be translated into clinical trials to assess the therapeutic potential of gene therapy for liver diseases and other regenerative medicine applications.

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