Generating Hepatic Spheroids to Study the Role of YBX1 in Regulating Inflammatory Signaling

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Abstract

Excess fat buildup in liver tissue can cause inflammation by activating signaling between liver and immune cells. This study investigates whether YBX1 regulates inflammatory signaling in hepatic spheroids under lipid stress. Human hepatic spheroids were cultured and exposed to a fatty acid mixture (oleate and palmitate) to mimic high-fat conditions. YBX1 expression was silenced using siRNA to evaluate its regulatory role. Gene expression was measured using mRNA analysis to assess changes in inflammatory signaling. It is expected that YBX1 silencing will alter inflammatory gene expression in hepatic spheroids exposed to fat. This will help determine whether YBX1 plays an important role in regulating liver inflammation. These findings may improve understanding of liver inflammation and help identify new targets for treating fatty liver disease.

Background

- Excess dietary fat → lipid buildup in liver cells (hepatocytes) → steatosis (fatty liver), inflammation, and possible fibrosis (liver scarring).
- Hepatocytes communicate with immune cells through cytokines (inflammatory signaling proteins).
- YBX1 = transcriptional/RNA-binding protein that may regulate inflammatory gene expression.
- YBX1 is hypothesized to function as a central regulator of lipid-induced inflammatory signaling in hepatocytes.

Background (continued)

- Hepatic spheroids are 3D liver culture models that better preserve cell-cell interactions and tissue-like architecture than standard 2D culture.
- Multicellular spheroids can combine hepatocyte, stellate, and immune-like compartments in one system, making them useful for modeling steatosis, fibrosis, and stress responses.
- These models are useful for studying long-term lipid exposure because they can capture morphology and lipid-handling phenotypes in a more physiologic format.
- A lab-built tri-culture spheroid system could provide a practical platform for testing whether chronic dietary stress drives inflammatory or fibrogenic responses.

Methods

- Prepared HepG2 and LX-2 cells as single-cell suspensions; differentiated THP-1 cells into macrophage-like cells with PMA before spheroid assembly.
- Mixed cells at two tri-culture ratios: 10:1:1 and 5:2:1 (HepG2:LX-2:THP-1).
- Seeded 100 µL/well into ultra-low-attachment 96-well plates and incubated at 37 °C, 5% CO2.
- Monitored spheroid formation over 24–48 h; added low-percentage Matrigel support after compaction in selected wells.
- Maintained cultures with gentle media changes to avoid disrupting spheroid structure.

Results

- We were successful in generating multicellular liver spheroid-like structures in culture.
- The aggregates appeared round, compact, and visually consistent with spheroid formation.
- Spheroid formation was observed across the tested tri-culture conditions, indicating that the assembly workflow is feasible in our lab.
- These data support successful establishment of the initial hepatic spheroid platform.

Conclusions

The current data support successful spheroid formation, but not yet a confirmed functional immune or fibrogenic response. More testing is required to determine whether these spheroids mount an immune response to long-term lipid stress.

Next Steps

- Use spheroids to study liver–immune interactions in vitro.
- Induce lipid stress with free fatty acids (oleate + palmitate).
- Knockdown YBX1 using siRNA.
- Measure gene expression to assess gene x environment interactions.

References

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