Targeting the TIMPs with PROTAC-based small molecules as a potential therapeutic approach for Liver cirrhosis treatment.

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Liver cirrhosis is a common problem seen during liver injury and scars by toxic materials such as alcohol, etc. During chronic tissue damage in the liver, for example: Hepatitis, continuous hepatocyte cell death leads to the activation of the hepatic stellate cells (HSC) which are responsible for fibrosis eventually resulting in cirrhosis. It has been previously shown that the tissue inhibitors of metalloproteinases (TIMPs) 1 & 2 secreted by the activated HSCs prevent the resolution of liver fibrosis. In this study, we hypothesize that by selective degradation of TIMPs 1 & 2 through PROTAC-based small molecules, one can selectively degrade the TIMPs thus helping the matrix remodelling to reinitiate and clear the fibrosis in order to prevent liver cirrhosis.

Keywords: Liver cirrhosis, PROTAC, MMPs, TIMPs, TIMP inhibitors.

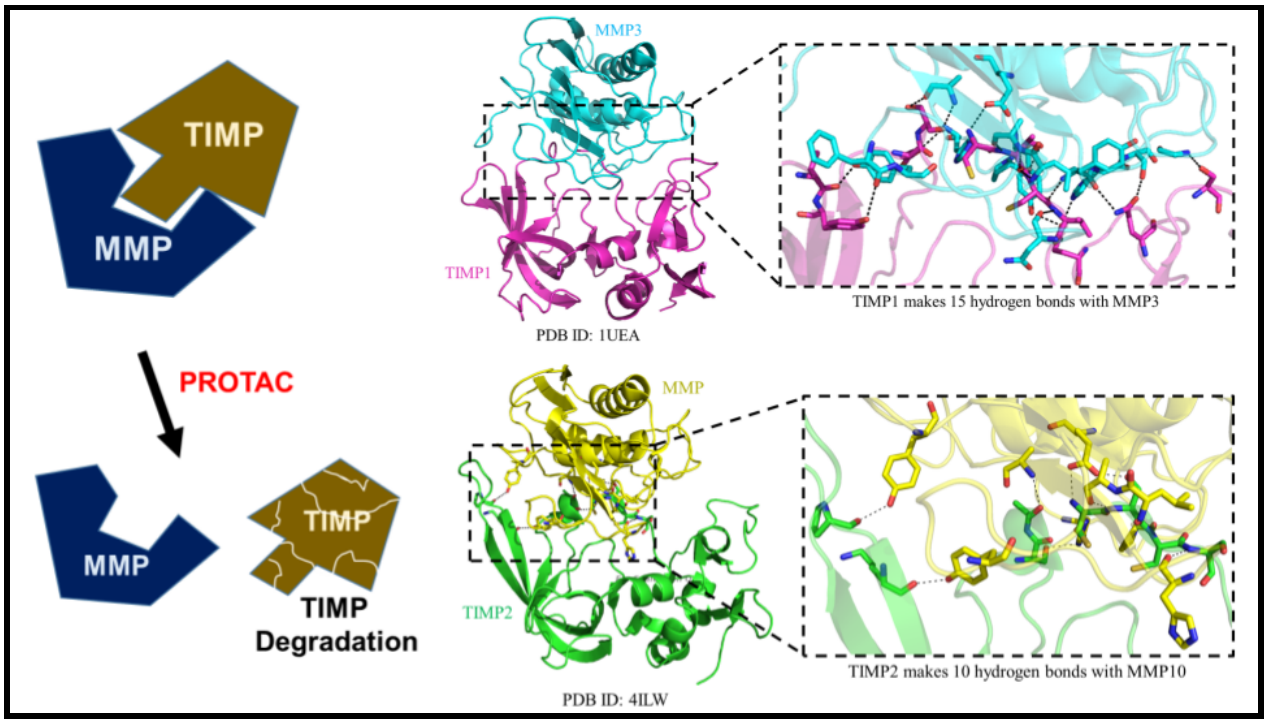

Figure 1. Overall strategy to target TIMPs with PROTAC shown on the left with molecular details of TIMPs 1/2 on the right.

Liver Cirrhosis (LC) is a common problem seen during liver injury which causes scars. Injury can be caused by various toxic substances such as xenobiotics, alcohol, etc. Biopsy may cause complications so ultrasound technique is commonly used. Increased fat deposits result in liver steatosis due to obesity and alcohol intoxication. Steatosis can often lead to hepatic disorders such as type-2

diabetes. During chronic tissue damage in the liver, for example: Hepatitis, continuous hepatocyte cell death leads to the activation of the hepatic stellate cells (HSC) which are responsible for fibrosis eventually resulting in LC. This is a long term liver damage that goes through various stages mostly due to remodeling and formation of scar tissue. During fibrosis or LC, due to altered architecture of extracellular matrix within the liver, the hepatic portal veins may get squeezed causing a blockage in the blood flow. This results in portal hypertension that is commonly seen in LC. The activated HSCs can either go back to the resting stage or undergo apoptosis resulting in the resolution of the scar tissue without progressing into fibrosis. However, depending on the extent of the liver damage, the activated HSCs may progress towards excessive fibrosis and also chemotaxis thus spearing the fibrosis in the surrounding healthy hepatic tissue. Some of the complications include gastrointestinal bleeding, weakness, fatigue, abdominal swelling and jaundice. In some cases LC may lead to hepatocellular carcinoma. The progressive damage beyond LC cannot usually be reversed.

During the progression towards fibrosis, the extracellular TIMPs (tissue inhibitors of metalloproteinases) are upregulated which inhibit the extracellular matrix metalloproteinases (MMPs). MMPs are involved in remodeling the extracellular matrix but in fibrosis, due to their inhibition by the TIMPs, the excess fibrosis leads to increased scars resulting in the LC. The activated HSCs may sometimes lead to resolution of the fibrotic tissue through their senescence (HSC reversion) or apoptosis. But unfortunately, the process of liver damage is irreversible beyond LC. Currently, there are no treatments for LC and new therapeutic approaches are needed to explore the MMP-mediated extracellular matrix remodeling in fibrosis and the role of TIMPs in inhibiting the MMPs in the context of LC. Prevention is better than cure. Alcohol consumption might be controlled to reduce liver damage but the viral infection-mediated scars cannot be avoided all the time. Also, better and more sensitive methods of diagnosis are also required to diagnose the liver damage in early stages so that the extracellular tissue remodeling can be controlled much in advance before going to the LC stage of the damage.

In this study, we hypothesize that by using small molecules one can selectively target the TIMPs 1 and/or 2 for degradation by the host ubiquitin-proteasome system. The small molecules act as PROTACs (proteolysis targeting chimera) in which one part binds to the TIMPsv1 or 2 and the other part binds to the host ubiquitin E3-ligase enzyme such that the PROTAC brings the host ubiquitin E3-ligase enzyme to the vicinity of TIMPs and thus ubiquitylating them towards proteasomal degradation. In order to understand the overall organization of the TIMPs 1 and 2, the 3-dimensional analysis of their structures was performed using Computational Biology tools. Structures were downloaded from the protein data bank (PDB IDs: 1UEA and 4ILW). Secondary structure analysis: The analysis includes evaluation of the secondary structure (alpha-helices and beta-strands) using PyMOL molecular graphics software. The amino acid sequence of the protein was shown in the display and was color coded by the secondary structure. Then the color coded domains were counted and analyzed accordingly for secondary structural elements. Hydrogen bonding analysis was performed using PyMOL molecular graphics software. The polar contacts option was turned on and all the polar contacts less than 3 Å were considered as strong hydrogen bonds which were listed and analyzed further.

The structures of TIMP1 and TIMP2 (Figure 1) in complex with MMPs revealed multiple hydrogen bonds in the binding

interface. As shown in Figure 1, TIMP1 interacts with MMP3 with 15 hydrogen bonds indicating a strong interaction. In order to prevent fibrosis, the MMP must be released from TIMP1. However, with 15 hydrogen bonds at the interface, it will be hard for a traditional reversible inhibitor to break the interaction of TIMP1 with the MMP. Hence, PROTAC design was chosen to degrade the TIMP1 altogether instead of breaking its interaction with the MMP. TIMP2 shows more of an open conformation compared to TIMP1 which may or may not be relevant to this study. As shown in Figure 1, the amino acids from TIMP2 involved in binding interactions with MMPs were analyzed in detail to understand the type of bonding. Evidently, due to its open conformation, TIMP2 shows less number of hydrogen bonds compared to TIMP1. Ten hydrogen bonds were seen between TIMP2 and MMP10 (Figure 1). Also, the open conformation of TIMP2 provided extra sites to explore for PROTAC design such that no steric hindrance from the MMPs is going to affect its binding to the TIMP2. We are currently in the process of synthesis and evaluation of the inhibitors that will be evaluated *in vitro* and *in vivo*. All data obtained from the inhibitor evaluation will be published in the future issues of TCABSE-J.

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Conflict of interest: The strategic communication presented here is an ongoing project currently at TCABS-E, Rajahmun--dry, India. The authors invite collaborations without any conflict of interest.