Heat-shock proteins (HSPs) are molecular chaperones highly expressed in hematological malignancies. They promote proliferation, immune escape, and resistance to chemotherapies. Our team and others identified HSP110 as a pro-survival factor in diffuse large B-cell lymphomas (DLBCL). In particular, HSP110 sustains aberrant NFkB signaling in activated B-cell DLBCL (ABC-DLBCL) through MyD88 stabilization (Boudesco et al. Blood 2018 Aug 2;132(5):510-520). These findings highlighted HSP110 as a new potential therapeutic target in DLBCL. However, the role of HSP110 in the global signaling pathway of DLBCL has not been studied yet. In addition, the impact of HSP110 inhibition in in vivo xenograft models has been hampered by the absence of specific inhibitors.

Aims:

We recently identified two foldamers by their capacity to specifically disrupt HSP110 functions in colorectal cancer models (Gozzi et al. Cell Death Differ. 2020 Jan;27(1):117-129). These first HSP110 inhibitors have given us the opportunity to gain more insight into the signaling pathways controlled by HSP110 in DLBCL in vitro and in vivo.

Methods:

We treated DLBCL cell lines in vitro, and in vivo in xenografted NSG mice. Analyses were done using Western blot, IHC, proximity ligation assay, and in vitro phosphorylation assay.

Results:

We first validated the two HSP110 inhibitors in DLCBL since they efficiently disrupted the binding of HSP110 to MyD88 in DLBCL cell lines. They impaired cell growth and led to cell death in the Myd88L265P cell lines OCI-Ly10, HBL1, TMD8, and OCI-Ly3 in vitro. Treatment of TMD8-xenografted NSG mice with increasing doses of HSP110 inhibitors limited tumor growth, without tissue toxicity. Analysis of xenografted tumors and in vitro treatments of DLCBL cell lines revealed that the MyD88/NFkB pathway was altered as expected, but also the BCR pathway since we observed a decrease in the activation of BTK, SYK, and PLCy2.

Using immunoprecipitations and Duolink™ assays, we identified an in vitro and in cellulo physical interaction between HSP110 and SYK (but not BTK) in several DLBCL cell lines. Furthermore, HSP110 inhibitors disrupted the binding of HSP110 with SYK in vitro, suggesting that the foldamers may act through this mechanism in vivo. We confirmed this assumption using Duolink™ assays, which showed fewer interactions in xenografted tumors after treatment.

Moreover, the phosphorylation of SYK recombinant protein is increased in vitro in the presence of a HSP110 recombinant protein, suggesting that HSP110 acts as a molecular chaperone for optimal activation of SYK through phosphorylation.

In addition to the MyD88L265P promotion of the NFkB pathway, the chronic BCR activation in DLBCL has been...
shown to mediate the activation of both the SYK/BTK and PI3K/Akt pathways, converging to NFkB. Targeting these pathways with ibrutinib and copanlisib, respectively, synergistically suppresses tumor growth in xenograft models (Paul et al. Cancer Cell 31, 64–78, January 9, 2017). We showed that the combination of HSP110 inhibitors with copanlisib also decreases SYK/BTK and AKT phosphorylation synergistically, leading to less NFkB activation in vitro and in vivo. Accordingly, the drug combination stopped the progression of the xenograft.

Summary/Conclusion:

In conclusion, we identified HSP110 as a regulator of BCR signaling through SYK chaperoning in DLBCL. This finding highlights the major potential of HSP110 as a therapeutic target in DLBCL and suggests a broader role in immune responses.