Smooth muscle cell responses in in-stent restenosis: the role of necroptosis

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Introduction

- Atherosclerosis – the build-up of plaque inside arteries – is the primary cause of death in the developed world [1]. The most popular form of treatment for atherosclerosis is the implantation of a stent, to reopen the artery lumen. This metal scaffold can fail (called restenosis) if plaques continue to build up, this failure can lead to many medical complications.
- Necroptosis, which is a form of cell death that causes inflammation, is thought to play a role in restenosis. The effect of necroptosis has been investigated in mouse models but never in human models; therefore, human models should be examined in order to achieve a more accurate depiction of the restenosis process.

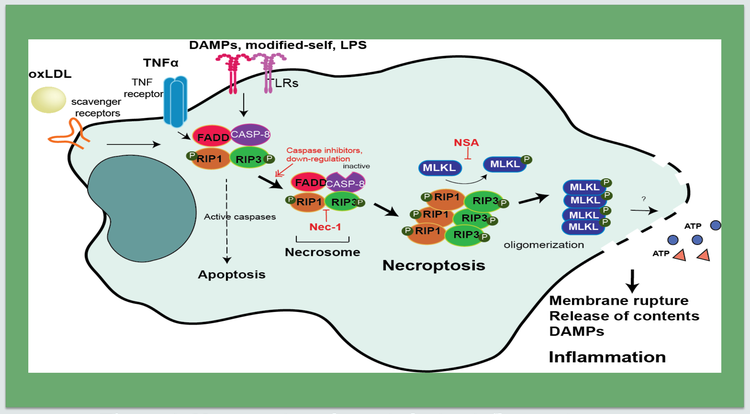

Figure 1. Necroptotic pathway inducing inflammation.

Methodology Part 1

Human smooth muscle cells (SMC) were plated in 24-well plates and treated for 24 hours with different combinations of necroptotic ligands and inhibitors.

After a 24h incubation, the media was collected and spun down to remove any cells debris. Cell lysate was collected for use in Western blotting.

10µL of supernatant was loaded into a 96-well plate with 200µL of LDH assay buffer and used in a 10 minute kinetic LDH (lactate dehydrogenase) assay.

The absorbance was measured at 305nm and cell death was determined from the change in absorbance over 10 minutes.

Results Part 1

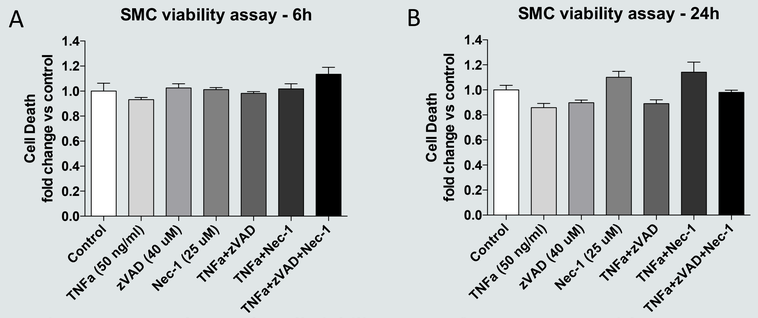

Figure 2. Smooth muscle cell viability assay after treatment with necroptotic ligands and inhibitors. Smooth muscle cell growth media was used in an LDH viability assay to test for necroptotic cell death **A)** after 6 hour treatment and **B)** after 24 hour treatment.

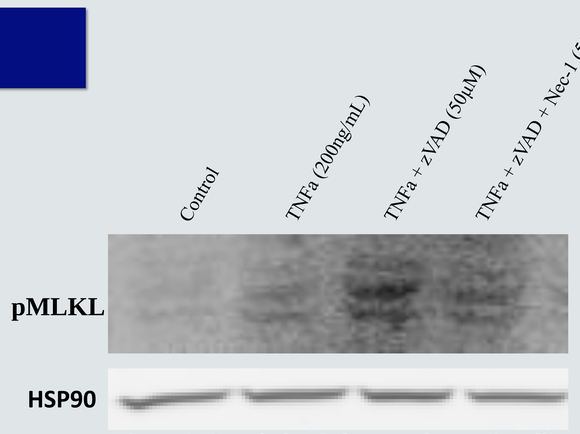

Figure 3. Protein levels in smooth muscle cells after 24h treatment with TNFa, zVAD, and Nec-1. Cell lysate was probed for pMLKL to observe if necroptotic ligands produce a change at the level of protein.

Methodology Part 2

Bare metal stents were immobilized on a collagen gel. One sample was not coated and the other was coated with 1mM Nec-1.

Smooth muscle cells were plated on the bare metal stent and allowed to grow and adhere to the stent.

The stent was stained with DAPI and imaged to observe cell growth on and around the stent.

Results Part 2

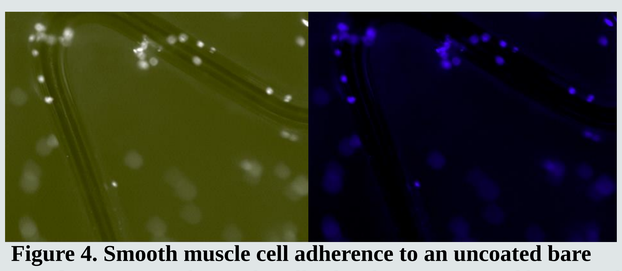

Figure 4. Smooth muscle cell adherence to an uncoated bare metal stent. Smooth muscle cells plated on an uncoated bare metal stent in DMSO as the vehicle control. The left image is taken with visible light spectrum and the right image was taken using DAPI staining.

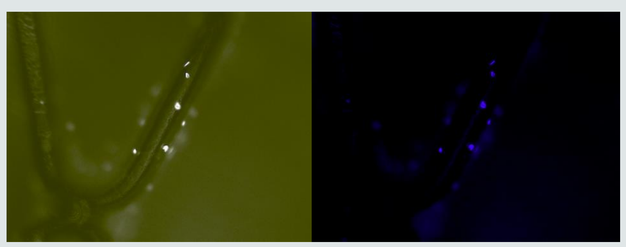

Figure 5. Smooth muscle cell adherence to a Nec-1 coated stent. Smooth muscle cells plated on a bare metal stent coated with 1mM Nec-1. The left image is taken with visible light spectrum and the right image was taken using DAPI staining.

Conclusion

It was initially predicted that the presence of necroptotic ligands such as TNFα would result in a significant amount of SMC necroptosis, however the results in Figure 2 refute this hypothesis. SMCs appear to be resistant to necroptotic cell death through a currently unknown mechanism. In contrast, Figure 3 shows an increased pMLKL levels in the cell lysate that leads to necroptotic cell death. The higher pMLKL levels could result in other cell types (macrophages, endothelial cells) undergoing necroptosis. The stent experiments proved that it is possible for SMCs to adhere to a stent that was coated in Nec-1, indicating that it is not completely toxic to cells and could potentially be used as a treatment to reduce restenosis rates.

Future experiments will include repeating the necroptosis assays using different cell types to see if TNFα will induce necroptosis in other cells present in the vessel wall, and repeating the drug coated stent experiment with lower concentrations of Nec-1 to determine the concentration at which cells are still viable.

References

[1] Merck Manuals Consumer Version, (2016) Atherosclerosis. [online] http://www.merckmanuals.com/home/heart-and-blood-vessel-disorders/atherosclerosis/atherosclerosis (Accessed March 4, 2016).
[2] Thermofisher.com, (2016) Pierce LDH Cytotoxicity Assay Kit - Thermo Fisher Scientific. [online] https://www.thermofisher.com/order/catalog/product/88953 (Accessed February 29, 2016).

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