

# **Index**

|  |     |
|--|-----|
| Zusammenfassung.....   | I   |
| Abstract.....  | II  |
| Index of abbreviations .....   | III |
| 1. Introduction .....  | 1   |
| 1.1. Pathology of vascular calcification in atherosclerosis .....                              | 1   |
| 1.2. Extracellular vesicles - vehicles for intercellular communication .....                   | 2   |
| 1.3. Calcifying extracellular vesicles – a specific subset? .....                              | 5   |
| 1.4. Phosphoinositides as fine-tune regulators of active cell membranes .....                  | 6   |
| 1.5. The endolysosomal system .....  | 8   |
| 1.6. Aim and objectives .....  | 11  |
| 2. Materials and methods .....   | 12  |
| 2.1. Material lists .....  | 12  |
| 2.2. Buffer and solutions.....   | 20  |
| 2.3. Methods .....   | 21  |
| 2.3.1. Cell biological methods .....   | 21  |
| 2.3.2. Molecular biological methods .....  | 24  |
| 2.3.3. Calcification detection methods.....  | 26  |
| 2.3.4. Extracellular vesicle isolation and detection methods .....                             | 27  |
| 2.3.5. Statistical analyses.....   | 29  |
| 3. Results .....   | 30  |
| 3.1. Calcifying conditions cause an increase of TNAP activity and calcification.....           | 30  |
| 3.2. Effectivity of apilimod stimulation in vascular smooth muscle cells .....                 | 31  |
| 3.3. Apilimod increases the release of extracellular vesicles with reduced TNAP abundance..... | 33  |
| 3.4. PIKfyve inhibition reduces collagen secretion and calcification.....                      | 34  |
| 3.5. PIKfyve inhibition reduces cellular TNAP.....   | 36  |
| 3.6. Cellular mechanism of TNAP reduction.....   | 38  |
| 3.6.1. Apilimod induced vacuole formation.....   | 39  |

|   |           |
|---|-----------|
| 3.6.2. Role of transient receptor potential mucolipin 1 activation in apilimod-mediated TNAP reduction.....               | 40        |
| 3.6.3. Role of phosphatidylinositol 3-phosphate in apilimod-mediated TNAP reduction .....                                 | 42        |
| 3.6.4. PIKfyve inhibition blocks late-stage autophagy .....   | 44        |
| 3.6.5. Identifying a transcription factor that mediates apilimod-induced TNAP reduction .....                             | 45        |
| 3.6.6. Role of sorting nexin 9 in apilimod-mediated TNAP reduction .....  | 46        |
| 3.6.7. Role of intracellular trafficking proteins in Aplimod-mediated TNAP reduction .....                                | 47        |
| 3.7. Separation and characterization of a calcifying extracellular vesicle fraction .....                                 | 49        |
| <b>4. Discussion.....</b>   | <b>52</b> |
| 4.1. TNAP as a marker of the calcification potential of vascular smooth muscle cells and extracellular vesicles.....      | 52        |
| 4.2. The role of endolysosomal maturation in vascular smooth muscle cell calcification.....                               | 53        |
| 4.2.1. PIKfyve inhibition alters the release of extracellular vesicles from calcifying vascular smooth muscle cells ..... | 53        |
| 4.2.2. Cellular mechanism of PIKfyve inhibition-mediated TNAP reduction .....   | 54        |
| 4.2.3. The role of autophagy in apilimod-induced TNAP reduction .....   | 56        |
| 4.2.4. The role of intracellular signaling in apilimod-induced TNAP reduction.....  | 57        |
| 4.3. Methods to isolate a calcifying extracellular vesicle subtype.....   | 59        |
| 4.4. Summary and Conclusion .....   | 61        |
| <b>References.....</b>  | <b>64</b> |
| <b>Appendix .....</b>   | <b>75</b> |
| <b>Publikationen.....</b>   | <b>76</b> |
| <b>Danksagung .....</b>   | <b>77</b> |
| <b>Eidesstattliche Erklärung .....</b>  | <b>79</b> |