Fetuin-a-based theranostics in ectopic calcification

7. SOCIETAL IMPACT

Ectopic calcification is defined as a pathological deposition of calcium salts in soft tissues. It accompanies a multitude of systemic diseases, with cardiovascular calcification in atherosclerosis and CKD being the most salient examples of the detrimental effects associated with this condition. Vascular calcification is strongly associated with all-cause mortality and long recognized as an independent risk factor for acute cardiovascular events, as it directly contributes to plaque instability in case of intimal calcification and arterial stiffness with subsequent left ventricular hypertrophy, MI and heart failure in medial calcification linked to end-stage renal disease [29, 174]. In fact, distinct forms of cardiovascular calcification are observed in 60-70% of individuals aged 41 to 80 years [175], and the prevalence increases up to 81% in patients with T2DM [176] and to a striking 100% in end-stage renal disease patients on hemodialysis [177]. As ectopic calcification is a feature of numerous diseases, it is difficult to assess its real burden. However, the very fact that conditions traditionally linked to extraosseous mineralization, such as IHD, stroke, diabetes, and CKD, are among the leading causes of global disability-adjusted life-years [178], clearly indicates the great impact of this health problem. Despite decades of research, there are no pharmacological treatments to completely stop progression of ectopic calcification, and the reversal of the process is considered the holy grail in the field of mineral pathophysiology. Moreover, the lack of sensitive and non-toxic imaging agents capable of detecting micrometer-sized calcified lesions creates additional obstacles in the management of the disease.

In this dissertation, I sought to develop a new approach to imaging and potential treatment of vascular calcification by establishing a novel theranostic platform based on mineral-binding protein fetuin-A. Firstly, I demonstrated that the chimeric proteins created upon fusion of fetuin-A and fluorescent protein variants were fully functional and could be used to detect mineralization and calcification in living cell cultures. Next, it was shown that fetuin-A-based probes surpass existing methods for detecting calcifications in both sensitivity and specificity. Application of novel imaging agents enabled identification of microcalcifications in seemingly intact tissues, significantly enhancing the diagnostic potential of optical microscopy in histological confirmation of soft tissue calcification, while eliminating the need for high-cost electron microscopy for early diagnosis of this pathology.

Further, radiolabeled fetuin-A was successfully used to visualize mineralized tissues in mice, demonstrating the potential of the molecule as a tomographic imaging agent. Finally, I proposed a new cell-based approach to the treatment of ectopic calcification based on the use of a chimeric cytokine that provides targeted activation of osteoclasts capable of resolving calcium salt deposits.

Taken together, the findings of this dissertation shed light on the application of bioengineered proteins to diagnose and treat mineral metabolism disorders. While some of the developed agents, such as fusion fluorescent proteins, do not require substantial optimization and can find immediate application in pathology departments, contributing to improved diagnosis of the condition, others might need further validation to advance the current management of ectopic calcification.