Intracellular cathepsin B inhibition with DARPin

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Cysteine cathepsins are endolysosomal proteases which over the recent years were established as key players in many intra- and extracellular physiological processes. Their intracellular role is often linked with cell death regulation following release into the cytoplasm, where they act as activators of regulatory proteins. However, their broad substrate specificity which is often overlapping between different members of the cathepsin family makes it hard to design specific inhibitors and substrates, as demonstrated by many failed attempts in clinical studies. Therefore, when elucidating cellular mechanisms it is important to critically interpret the results obtained with inhibitors which are generally considered specific but in reality are not, especially in high concentrations which are usually used when studying processes in cell culture. The use of intracellularly expressed specific binders has a potential advantage over traditional knock-out experiments and inhibitors, because they can be targeted to a specific location and therefore, do not disturb the physiological function of their target elsewhere. We, therefore, set out to compare an inhibitor considered specific for cathepsin B (CA074-OMe) with a cathepsin B specific DARPin (designed ankyrin repeat protein) expressed intracellularly.