Imaging approaches to understand the effects of bisphosphonate drugs on macrophages outside the skeleton

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Bisphosphonates (BPs) are a class of calcium-seeking drugs used clinically to inhibit bone resorption. BPs such as zoledronic acid (ZOL) act by inhibiting FPP synthase, (an enzyme of the mevalonate pathway) in osteoclasts, preventing the prenylation and thereby disrupting the function of small GTPase proteins that govern cytoskeletal dynamics and bone resorption.

Accumulating evidence suggests that BPs have pleiotropic effects outside the skeleton including anti-tumour activity and decreased mortality. However, the basis for these additional effects are poorly understood. Our findings suggest that these pleiotropic effects may be mediated by targeting soft tissue macrophages. We have shown that intravenous or subcutaneous administration of ZOL inhibits the prenylation of Rab GTPases in mouse peritoneal macrophages *in vivo*. Using 2-photon intravital imaging of fluorescently-tagged BPs, we also discovered that BPs accumulate in lymph nodes and are internalised by sub-capsular sinus macrophages. Similarly, we demonstrated that BPs diffuse from leaky vasculature in 4T1 mammary tumours and accumulate in the stroma by binding to microcalcifications. These microcalcifications are then engulfed by tumour-associated macrophages (TAMs), but not by tumour cells, suggesting that the anti-tumour effect of BPs is mediated indirectly by altering the function of TAMs, rather than affecting tumour cells directly.

Live cell imaging and immunofluorescence staining of IC-21 immortalised mouse macrophages revealed that treatment with ZOL impaired cell adhesion (~50%), motility (<60% speed and displacement), and caused loss of morphological polarisation characterised by the absence of clear leading (lamellipodia) and lagging (uropod) ends, without compromising cell viability. Importantly, these defects in macrophage morphology and migration were proportional to the accumulation of unprenylated proteins, and restoration of protein prenylation by supplementing cells with the missing lipid metabolite completely rescued cell morphology and cell migration.

Using a combination of advanced imaging approaches our findings challenge the long-held notion that BPs only target the skeleton, and demonstrate that these drugs can affect tissue resident macrophages outside bone, potentially altering innate immune function.

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