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Monoclonal Gammopathies & Multiple Myeloma

IDENTIFICATION BY A SINGLE CELL ANALYSIS OF CCN5/WISP2 AS A POSSIBLE MARKER OF THE OSTEOGENIC IMPAIRMENT IN THE MYELOMA BONE MICROENVIRONMENT

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Introduction: In multiple myeloma (MM), bone disease arises from an imbalance between bone formation and resorption, leading to osteolytic damage and the establishment of a tumour-permissive bone microenvironment (BME). Evidence suggest that an increase of bone resorption process occurs in monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM), whereas the disruption of the osteoblastic niche characterizes the progression to MM. Recently our group using a single-cell atlas of osteoblastic lineage cells have begun to describe the BME of MM patients and its precursors disease to identify possible markers of bone disease and tumoral progression. Thus, in this study we investigated the possible role of CCN5/Wisp2, a regulator of Wnt and TGF-β signalling, in the osteogenic differentiation process and in osteoblast dysfunction in MM as compared to precursors diseases.

Methods: Bone cells from 16 bone biopsies from MGUS, SMM, or newly diagnosed MM patients were analysed by single-cell RNA sequencing. For *in vitro* validation, hTERT-MSCs and 17 primary MSCs (SMM=8 and MM=9) were differentiated to OBs for 21 days with ascorbic acid and dexamethasone. Osteogenic commitment and *CCN5* expression were assessed by qPCR. To test the influence of MM-derived factors, differentiating cultures were exposed to conditioned media (CM) from human myeloma cell lines (HMCLs).

Results: scRNA-seq profiling of 42,823 BME cells identified 14 transcriptionally distinct to mesenchymal to OBs clusters. *CCN5* expression increased progressively along the osteogenic differentiation, from proliferating/immature MSCs and peaking in pre-OBs, OBs, and also in one adipocyte-like cluster. Stage-wise comparison revealed significantly higher *CCN5* expression in MGUS than in MM pre-OBs and OBs, suggesting an early activation of this pathway that decreases with disease progression. Notably, pre-OBs and OBs from non-progressor SMM patients displayed significantly higher *CCN5* levels compared to those from SMM-progressors. *In vitro CCN5* was strongly upregulated during osteogenic differentiation of hTERT-MSCs, with expression peaking at days 7 and 14. In primary patient-derived MSCs, *CCN5* expression was significantly upregulated at day 21 in SMM-derived, but not in MM-derived OBs. Exposure to CM from 2 HMCLs (JJN3 and OPM2) significant downregulated *CCN5* in hTERT-derived and SMM-derived OBs, whereas MM-derived OBs were largely unresponsive. Ongoing work includes immunohistochemical localization of CCN5 in bone biopsies and functional modulation through viral-mediated knockdown or overexpression.

Conclusions: By integrating single-cell and functional analyses, our data suggest that *CCN5* upregulation could characterize an osteogenic program preserved in MGUS and SMM non-progressors but progressively suppressed by myeloma-derived factors. Overall, these data identify *CCN5* gene as a potential biomarker of the osteogenic impairment that occurs in MM patients.