## Angiopoietin expression by human myeloma cells

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Uneda S et al. reported in issue 88 of Haematologica[1] that human myeloma cells express angiopoietin-2 (Ang-2) but not angiopoietin-1 (Ang-1) other than VEGF. The authors showed that KHM-11, ARH-77 and 6 out of 10 primary multiple myeloma (MM) cells express Ang-2 as well as one MGUS patient. On the contrary all the samples tested were negative for Ang-1. These observations on angiopoietin system expression by myeloma cells are in contrast with our recent evidence indicating that several human myeloma cell lines (HMCLs) and fresh purified MM cells obtained from 11 out of 23 patients express Ang-1 but not Ang-2 mRNA and protein.[2] We have some doubts about the methodology used by the authors. First, they did not purify bone marrow (BM) samples by a positive selection with anti-CD138 microbeads coated Ab as widely accepted in the literature[3-4] but, on the contrary, they used negative selection with anti-CD2, CD3, CD4, CD14, CD19, CD66b and glycophorin A-conjugated immunomagnetic beads. This type of selection cannot exclude the presence of endothelial cells in the samples that can be positive for Ang-2.[2,5] In addition, they used a primers pair for Ang-2 (as well as for Ang-1) that does not span any intron region but it is localized in the same exon region (the first one for Ang-2) with a high probability of false positive results due to DNA contamination in RNA samples. Immunohistochemistry data, showed by the authors, indicate that the EBV positive cell line ARH-77 and the HMCL KHM-11 express Ang-2. Since we failed to detect Ang-2 in ARH-77 and in HMCLs U266, RPMI-8226, OPM-2, XG-1, XG-6 we would like to know whether a negative control was used and whether other HMCLs were tested by the authors.

The authors also report the lack of Ang-1 expression by MM patients as well as by ARH-77 and KHM-11. It would be important to know at which stage of disease MM patients were tested and whether the authors evaluated Ang-1 protein expression in primary MM cells and in HMCLs other than ARH-77, which we also found negative for Ang-1 expression in contrast to several HMCLs.

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