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Confrontation cultures of embryonic stem cell-derived embryoid bodies and multicellular tumour spheroids: a novel *in vitro* model for the study of tumour-induced angiogenesis

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Background: Vascularization is a prerequisite for tumour growth and metastasis. Therefore anti-angiogenic therapy is one of the most promising strategies to defend against cancer. Consequently a large number of anti-angiogenic agents is currently tested in animal experiments that include the rabbit corneal assay, the mouse cheek pouch assay, and the mouse and rabbit cranial and skin window. Anti-angiogenic substances, such as thalidomide, are nowadays routinely used in several cancer therapies, for example the treatment of multiple myeloma. Preclinical studies of such substances require a large number of animal experiments. Here we introduce an *in vitro* model for anti-angiogenic screening based on co-culturing of embryonic stem cell-derived embryoid bodies and multicellular tumour spheroids, in order to reduce the number of animals needed for *in vivo* testing.

Methods: Embryonic stem cells and tumour cells were cultivated in separate spinner flasks to form 3-dimensional cell aggregates which are called embryoid bodies (EBs) or tumour spheroids respectively. 4 day old EBs were brought in close contact with tumour spheroids using the hanging drop technique. Once the aggregates grew together, they were transferred into bacteriological dishes or plated on gas-permeable cell culture dishes, and cultivated for additional time needed for the experimental setup. Analyses were performed using confocal laser scanning microscopy and the Leica analysis software, as well as flow cytometry and the CellquestPro software.

Results: We observed that blood vessels produced in co-culture are denser than in EBs grown alone, and also show directed growth of vessels towards the tumour. The anti-angiogenic and dose-dependent effect of three substances (thalidomide and the tyrosine kinase inhibitors SU5614 and ZM323881) was shown by immunocytochemistry and flow cytometry, based on staining for PECAM-1 (CD31) and VE-Cadherin (CD144). FACS analyses indicated that the number of CD31+ cells was not significantly changed, but the degree of vascularization was reduced upon treatment with anti-angiogenic agents. It is also shown that tyrosine kinase inhibitors have anti-inflammatory properties besides being anti-angiogenic, which was visualised by staining for leucocyte specific markers CD45, CD68 and neutrophil antigen. Preventing inflammation is of importance, since it often occurs next to the tumour, thus inducing a pro-angiogenic micro-environment.

Discussion: The EB derived from embryonic stem cells mimics early stages of embryogenesis, facilitating research on various differentiation processes of all three germ layers. This system combined with multicellular tumour spheroids simulates interactions between healthy and pathological tissue, thus mimicking the process of tumour-induced angiogenesis. In summary we demonstrate that confrontation cultures of EBs and multicellular tumour spheroids are suitable to replace or reduce the number of animal *in vivo* studies, e.g. the rabbit cornea assay, the hamster cheek pouch assay, the cranial window assay or the skin chamber assay. Our novel *in vitro* confrontation culture model can be used for screenings of various substances as well as for examinations of signalling pathways involved in host tissue-tumour interaction.

References

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