Integrin antagonistic drugs reveal different effectiveness in 2D monolayer vs. 3D spheroid culture

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Objective
Preclinical evaluation of novel therapeutic substances, as well as the assessment of radiation effects, is frequently performed under standard 2D cell culture conditions. However, such monolayer cultures may fail with regard to representation of morphological in vivo conditions and their biological consequences. An alternative is the use of 3D in vitro models – like tumor spheroid culture – which are of intermediate complexity between standard in vitro monolayer cultures and in vivo tumor models. In spheroid culture, tumor cells grow in 3D aggregates that display greater similarity to in vivo tumor architecture and growth conditions, such as the presence of oxygen and nutrient gradients as well as more complex cellular interactions or ‘in vivo-like’ gene expression profiles. Depending on their size, multicellular spheroids may also display central hypoxic and/or necrotic areas and show quiescent and proliferating compartments. Thus, spheroids often depict different behavior and sensitivity towards certain drugs or radiotherapeutic treatment as cells cultured as 2D monolayers. Especially for the study of surface receptors like integrins the 3D structure and environment is a critical aspect as these receptors transduce signals from the extracellular to the intracellular space, thus influencing different cell signaling pathways that control cell survival, proliferation and invasion.

Materials and Methods
Therefore, in addition two standard 2D cell culture. 3D spheroid models were established with the melanoma 518A2 and other cell lines for evaluation of their response to two different integrin antagonists, cilengitide and a novel integrin antagonist (NIA). In addition, we investigated the effects of radiation treatment alone or in combination with the drugs.

Results
While in 2D cultures of 518A2 melanoma cells, the comparator substance cilengitide showed to be more efficient IC50 value of 0.65µM than our novel compound NIA, cilengitide had no inhibitory effect in 3D spheroid culture up to 50µM. Comparatively, NIA had similar effectiveness in 2D as well as 3D cultures, both in the low micromolar range. During monitoring of spheroid growth, NIA treated spheroids initially depicted a growth retardation, before cells started to disintegrate and die. The radiosensitivity of 518A2 melanoma cells was found to be similar in both culture conditions.

Discussion
Similar differences in drug response and efficacy between 2D and 3D cell culture environments have been reported for various anti-cancer substances as well as for some radiation exposure endpoints. However, other endpoints may in a treatment-related manner – depend on the culture system used. We thus plan to perform further comparative studies on survival-dependent aspects (apoptosis, intracellular signaling, and others) with integrin antagonists alone as well as in combination with irradiation in 2D cell culture versus 3D spheroids.

Summary
• Cilengitide is effective in 2D 518A2 melanoma cell culture (and other cell lines; data not shown). The effect is less pronounced in spheroids (no inhibitory effect up to 25 µM).
• The novel integrin antagonist (NIA) has a similar activity in 2D and 3D cultures of 518A2 cells. Spheroids treated with NIA initially show a growth inhibition, before disintegration and cell death is induced.
• Radiosensitivity of 518A2 melanoma cells is similar under 2D and 3D culture conditions.
• The combination of NIA and irradiation has additive effects.