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Development and characterization of a novel, simple, spheroid-based tri-culture model composed of fibroblasts, keratinocytes, and melanoma cells

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Melanoma is the most common form of cancer. Thus, test systems for the development of new drugs and therapies are required that resemble the *in vivo* situation. Currently, most cell-based assays are performed in two-dimensional (2D) cell cultures. While cells cultured in 2D monolayers appear to be flat and stretched allowing for equal exposure to medium and drugs, three-dimensional (3D) structures of cells are more realistic to the *in vivo* situation displaying cells of varying stages of the cell cycle, i.e. proliferating cells on the periphery of the 3D structure, followed by quiescent and necrotic cells in the center. Several 3D cell culture approaches that exhibit varying degrees of complexity have been developed to perform drug testing and mechanistic studies on melanoma. Although 3D cell culture systems of melanoma are superior to traditional 2D approaches, these 3D cultures are either composed of only one cell type, the melanoma cells, or they are so complex that it is challenging to understand the behavior of individual cell types. Additionally, they are often difficult to establish and expensive. Therefore, this work aimed to establish a novel, simple, spheroid-based melanoma model. This study used low-attachment plates to generate spheroids that are composed of up to three different cell types, i.e. stromal, skin, and cancer cells. To study differences in culture conditions and behavior of normal and neoplastic cells, 3D cultures of these cell types were compared. 3D cultivation revealed decreasing volume sizes of normal cells, fibroblasts and keratinocytes, with only few proliferating cells on the periphery of the spheroids. In contrast, neoplastic cells, SK-MEL-28, LNCaP, and PC-3 cells, displayed increasing volume sizes with proliferating cells on the outside of the spheroids. All tested cell types showed a significant reduction of proliferation with a concurrent rise of apoptosis in 3D compared to 2D cultures. The presented tri culture model allowed the study of cellular behavior in a cell-type specific way and represented different features of early melanoma stages. Fibroblasts formed a collagen IV rich center of the tri-culture spheroid, keratinocytes built up a ring around this center, and melanoma cells arranged highly proliferating clusters on the outside. Some melanoma cells were also found regularly in the fibroblast core. In the absence of melanoma cells, keratinocytes stratified into an inner basal-like ring and more differentiated cells on the periphery. In contrast, addition of melanoma cells clearly reduced keratinocyte differentiation. Upon treatment with the cytostatic drug, docetaxel, this keratinocyte differentiation was restored and apoptosis of external melanoma cells was induced. Furthermore, docetaxel treatment significantly increased the amount of immunoreactivity to the transporter protein ABCB5 in remaining intact external melanoma cells. Taken together, a novel, simple, spheroid-based melanoma tri-culture model composed of fibroblasts, keratinocytes, and melanoma cells was described. This can now be applied for the development of new drugs and the analysis of their cell type specific mode of action.