

Summary

This Ph.D. thesis summarises the results of our research into the alterations observed during the differentiation of skeletal muscle cells and the malignant transformation of human melanocytes.

We have shown that when C2C12 mouse myoblasts and primary cultured mouse skeletal muscle cells are induced to differentiate by the appropriate alteration of the serum content of their culturing medium, the responsiveness of cells to extracellular ATP increases and the Ca^{2+} transients as well as the underlying Ca^{2+} fluxes become biphasic. The early peak component is initiated by the opening of ionotropic P2X receptors, especially the P2X₇R, followed by inward ionic currents, depolarization of the cell, initiation of an action potential and thus the activation of voltage-gated processes. This phase is sensitive to the removal of extracellular Ca^{2+} ions, to preceding and sustained membrane depolarization and to the inhibition of P2X receptors. Its kinetic parameters resemble those of the depolarization-induced Ca^{2+} transients, and it is rapidly terminated as voltage-gated channels inactivate. On C2C12 cells this early peak component appears only at the most differentiated stage, but if differentiation is induced by the overexpression of PKC α , the early phase fails to appear even in these cells (whereas the delayed phase can be detected with maximal amplitude early in differentiation). In the latter group of cells, the increase in the expression of P2X₇R is also lacking and the responses of cells resemble the transients of the ones that have undergone transfection with anti-P2X₇R siRNA displaying a suppressed early phase, which underlines the importance of the P2X₇ subtype during the peak component. In the case of primary cultured skeletal muscle cells, the biphasic shape of the Ca^{2+} transient appears already at an early stage, but, as a sign of the involution of purinergic signalling, the large peak disappears in the most developed myofibres and only the sustained phase can be detected. The latter component of the transient is produced by the release of Ca^{2+} from the SR following the activation of metabotropic P2 receptors (P2Y₂R and P2Y₄R on C2C12, while P2Y₁R and P2Y₄R on primary myotubes).

As compared to control healthy melanocytes, ATP sensitivity appeared in each of our melanoma cell lines as a gain of new function. Our experiments have shown that all the three melanoma cell lines express the P2X₇R, which, localised in the cytoplasmic membrane and having the typical pharmacological characteristics of P2X₇ receptors, is responsible for the ATP-evoked Ca^{2+} transients. Unlike in other tissues, however, the activation of the receptor exerts antiapoptotic effect, while its inhibition promotes apoptosis and necrosis *in vitro* and inhibits metastasis formation *in vivo*. The altered function of P2X₇R can be related to the overexpression of the ryanodine receptor in melanoma cells. We have demonstrated that RyR is localised in the ER as well as in the cytoplasmic membrane. Its well-known agonists are unable to activate the receptor, but ryanodine significantly decreased the amplitude of the P2X₇R-mediated Ca^{2+} transients. It cannot be excluded that the pathologically overexpressed RyR modifies the function of the P2X₇R in a way that the latter becomes a protective factor for the cell, thus leading to malignant transformation.

The results of the work discussed in this dissertation prove that diverse cell types make use of the purinergic signalling pathway as the regulator of processes critical in the determination of the fate of the cell, such as differentiation to a specialised function or avoiding apoptosis. We have also demonstrated that the P2X₇ receptor subtype is a key player in directing cells between proliferation and differentiation, as well as apoptosis and survival.