EFFECTS OF RGD LIGANDS ON INTEGRIN-MEDIATED ADHESION, SIGNALING AND TRAFFICKING IN CANCER CELLS

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Integrins are α/β heterodimeric transmembrane receptors that mediate cell-cell and cell-ECM interactions by binding various components of the extracellular matrix (ECM) or soluble ligands. As important sensors of the cell microenvironment, integrins regulate crucial aspects of cellular functions and have been implicated in various diseases including cancer, tumor metastasis, inflammation, thrombosis and autoimmune diseases. Among integrins, α5β1 integrin is over-expressed in novel vessel surrounding tumor cells and has been implicated in tumor development and cancer cell invasion. α5β1 integrin is internalized, trafficked to recycling endosomes and then recycled to the plasma; it has been demonstrated that these membrane trafficking pathways influence α5β1's capacity to promote invasion and metastasis. To date several monoclonal antibodies and small molecules targeting integrins have been approved for the treatment of multiple sclerosis, Chron's disease and thrombosis.

The functionality of receptor proteins depends directly upon their expression level on the plasma membrane. Therefore, the ability to selectively modify the surface level of a particular receptor is pivotal to many cellular signaling events. Dynamic remodeling of adhesions, through rapid endocytic and exocytic trafficking of integrin receptors, is an important mechanism employed by cells to regulate integrin-ECM interactions, and thus cellular signalling, during processes such as cell migration and invasion. The initial concept of integrin traffic as a means to translocate adhesion receptors within the cell has now been expanded with the growing appreciation that traffic is intimately linked to the cell signalling apparatus. Furthermore, endosomal pathways are emerging as crucial regulators of integrin stability and expression in cells and integrin traffic is relevant in a number of pathological conditions, especially in cancer.

Therefore, considering integrins as valuable drug target, the current study was performed to elucidate the effects of small molecules on integrin-mediated adhesion, signaling and trafficking. These new ligands, deriving from two chemically different libraries of peptidomimetics, were specifically designed to target RGD integrins for clinical application such as cancer. The biological activities of these new compounds were evaluated by investigating their effects on integrin-mediated cell adhesion in a suitable cell line (K562 cells endogenously expressing α5β1 integrin). Furthermore, their effects on cell signaling activated by integrins were investigated through the study of ERK phosphorylation by western blot, as the binding of endogenous ligands to integrins recruits several cellular components and modulates intracellular signaling cascades, especially those leading to the activation of FAK and MAPK pathways that play a crucial role in the regulation of numerous cell functions. In order to investigate the effects of the most effective peptidomimetics on integrin trafficking upon exposure to our ligands, α5β1 integrin internalization was analysed by confocal microscopy on HEK293 cells transfected with a plasmid containing the coding sequence of α5 fused with the fluorescent protein EGFP.

We found selective and potent agonists and antagonists of α5β1 integrin, able to modulate cell adhesion, signaling and trafficking. Interestingly, we observed that α5β1 integrin agonists were able to activate integrin-mediated signaling and to induce α5β1 integrin internalization, while, on the other hand, antagonists prevented fibronectin-induced ERK activation and integrin internalization. Most effective compounds may represent leads for the development of new therapeutic agents: integrin antagonists may be employed as anti-cancer or anti-angiogenic drugs, while integrin agonists may be used as shuttles for selective delivery of therapeutic payloads and diagnostics.