

Dissecting the mechanism and functional landscape of cancer PD1 signalling in osteosarcoma.

MPharm Katarzyna Dziubek

Cancer treatment was revolutionised by immune checkpoint inhibitors (ICIs) targeting the PD1/PDL1 axis, which acts as a brake to the immune system. Despite therapeutic success, some patients do not respond to therapy or rapidly deteriorate due to an unclear mechanism. As reported for several types of tumours, PD1 receptor is not solely expressed on immune cells but also on cancer cells. Moreover, depending on the tumour type, it may act either as a promoter or tumour suppressor and was implicated as a mechanism of resistance to ICIs. The limited response to ICIs was as well reported in osteosarcoma. This study aimed to determine if cancer-PD1 may account for the limited response of osteosarcoma to ICIs and to characterize the functional role of PD1 protein and its interactions in osteosarcoma cells.

Our results demonstrated both surface and intracellular expression of PD1 protein in U2OS osteosarcoma cells. Strikingly, PDCD1 gene silencing significantly increased cell migration and viability. LC-MS/MS based proteomic analysis revealed that PD1 alterations markedly affected the proteomic landscape of U2OS cells. Large-scale data interpretation tools GO and GSEA strongly indicated that PDCD1 silencing leads to enrichment of proteins involved in processes such as cell growth, migration, and motility, corresponding to the cellular effects we initially observed. STRING interactome analysis of the most affected proteins revealed their role in mTORC1 signalling, cell and focal adhesion, and increased metastatic potential, implying that cancer-PD1 in osteosarcoma may act as a tumour suppressor.

While PD1 signalling pathway was well characterized in T cells, contradictory results are available regarding its role in cancer, and little is known about cancer-PD1 interactome. Our in-depth PD1 interactome studies performed with LC-MS/MS based proteomics, identified AXL (receptor tyrosine kinase UFO) as a novel PD1 binding partner. The interaction between PD1 and AXL was confirmed with PLA and Western Blotting. Molecular docking studies, used to characterize the interaction, further confirmed protein binding, and indicated that it takes place in their intracellular domains. Aligning with our experimental data, PD1 mutations in tyrosine phosphorylation residues did not abrogate PD1 binding with AXL. However, the in silico analysis demonstrated that depending on the mutation, the protein complex was supported by distinct bonds, suggesting varying strength and affinity of the interaction.

In summary, our data report the previously undiscovered functional expression of PD1 protein by osteosarcoma cells and provide valuable insight into the landscape of PD1 downstream signalling. PD1 interactome studies identified a novel interaction between PD1 and AXL. Strikingly, previous reports demonstrated the improved response to PD1/PDL1 ICIs in combination with AXL inhibitors, therefore, our studies may shed new light on the underlying mechanism. However, further studies on cancer-intrinsic PD1 are urgently needed to understand its therapeutic significance to improve safety and efficacy of immunotherapy.