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Activation of the 3-phosphoinositide-dependent kinase 1 through phosphorylation at serine 135 in cancer cells: Characterization of monoclonal antibodies as diagnostic and prognostic tools

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Invasion of healthy tissue and metastasis are major causes of death in cancer patients. These processes require mechanisms, allowing cancer cell growth and survival independently of extracellular signals. Stimulation of cell metabolism, growth, and survival are, among others, achieved through abnormal activation of the phosphatidylinositol-4,5-bisphosphate 3-kinase/ 3-phosphoinositide-dependent protein kinase-1/ protein kinase B signaling cascade. The 3-phosphoinositide-dependent protein kinase-1 plays a central role within this cascade by modifying multiple downstream targets. Recently, a new mode of intracellular stimulation has been identified. This regulation involves a loop-back mechanism, whereby protein kinase c eta, in a complex with the protein radixin, phosphorylates 3-phosphoinositide-dependent protein kinase-1 at serine 135, causing its activation independently of the cofactor phosphatidylinositol (3,4,5)-trisphosphate. In human brain tumor cells, but not in normal human cells or tissues, human 3-phosphoinositide-dependent protein kinase-1 likewise becomes activated through this phosphorylation.

To further evaluate this activating phosphorylation as a potential diagnostic/ prognostic marker of malignant disease progression, this work aimed at (i) the generation and validation of monoclonal antibodies recognizing the phosphorylated kinase in western blot analysis, immunofluorescence staining and paraffin sections and (ii) the screening of numerous tumor-derived cell lines and tissues samples for its occurrence.

In total, 175 monoclonal antibody candidates were characterized. Throughout the process, two candidates were selected for further application. With their support, 37 human tumor-derived cell lines and eight human brain tumor samples were investigated. The phosphorylation of 3-phosphoinositide-dependent protein kinase-1 at serine 135 was successfully revealed in 72 % of all tested cell lines and in 80 % of all investigated brain tumor samples. Astonishingly, the incidence was as high as 91 % in neural crest derived tumor cell lines (n = 22) and even higher in high-grade brain tumor resectates (100 %).

The obtained results indicate a correlation of the investigated phosphorylation to highly aggressive tumor entities, especially in neural crest-derived malignancies such as neuroblastoma and malignant melanoma.
These observations, and the fact that neural crest progenitors and certain tumor cells utilize the same stem cell programs (e.g. epithelial–mesenchymal transition) for migration or metastasis, led to the hypothesis of the investigated phosphorylation being part of a developmental stem cell pathway, hijacked by cancer cells in order to gain metastatic potential, growth-factor independency or resistance to apoptosis. As the role of the phosphatidylinositol-4,5-bisphosphate 3-kinase/ 3-phosphoinositide-dependent protein kinase-1/ protein kinase B signaling cascade in cancer stem cells is subsequently elucidated, emerging evidence confirms its importance for stem cell-like behavior. These findings go together with the observed high incidence of the investigated phosphorylation in glioblastoma cancer stem cells.

This work therefore urges the potential of 3-phosphoinositide-dependent protein kinase-1, phosphorylated at serine 135, as a diagnostic marker in highly aggressive tumor entities of the brain. However, further work is necessary to elucidate its role in other tumor entities, especially in cancer stem cells of other origin. Additionally, a possible involvement in developmental stem cell processes has to be investigated. Should the occurrence remain (post-embryonic) tumor-exclusive, it has the potential to serve as a target for new therapeutic approaches.

This would be of great interest, as 3-phosphoinositide-dependent protein kinase-1 inhibitors were already effectively used to reduce cancer cell growth and metastasis in different tumor entities, but their application, due to the physiological role of the kinase in non-transformed cells, is so far limited to *in vitro* use. If the incidence of the investigated phosphorylation remains cancer-exclusive, a specific therapy (e.g. T-cell-receptor-like antibodies) could bypass this difficulty and offer new perspectives in targeting high-grade gliomas or even cancer stem cells.