Immunogenic cell death in vitro supports the production of a dendritic cell-based anticancer vaccine

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Cancer associated fibroblast-derived integrin α11 regulates extracellular matrix remodeling and tumor progression in breast cancer

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CDK4 phosphorylation status and corresponding gene expression profile predict sensitivity to Palbociclib.

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Although the specific CDK4/6 inhibitor PD0332991 (Palbociclib) was recently approved by the FDA to treat advanced ER+ breast tumors, there is yet no reliable sensitivity prediction tool. Cyclin D-CDK4/6 are the first CDK complexes to be activated in G1 phase in response to oncogenic pathways. They phosphorylate and inactivate the central cell cycle/tumor suppressor pRb. CDK4 activity requires its binding to a cyclin D (*CCND1-3* genes) with which INK4 CDK4 inhibitors such as p16 (*CDKN2A-D* genes) compete. Although the assembly of the CDK4-cyclin D complexes was considered to be the main level of CDK4 activity control, we have shown that the activating T172-phosphorylation of CDK4 is actually the central rate-limiting event that initiates the cell cycle decision and signals the presence of active CDK4.

Here, using 2D-gel electrophoresis to separate the modified forms of CDK4, we found in breast cancer cell lines that only the CDK4 T172-phosphorylation correlates with the sensitivity to PD0332991. The only exception was in the rare case of combined CCNE1 amplification and CDKN2A loss wherein combination of PD0332991 with a CDK2 inhibitor is required to block entry in the cell cycle. Additionally, three types of CDK4 modification profile were identified by 2D-gel electrophoresis in 56 breast tumors. In the first profile, the phosphorylated CDK4 was undetectable as in normal breast samples despite a high KI67 index. In the second and third profiles, the CDK4 phosphorylation was detectable and its intensity was either above or below 90% of the intensity of a second yet unidentified form of CDK4, respectively. The proportions of these profiles differ among breast tumors according to their clinic-pathological characteristics, molecular subtypes and risk. Finally, we identified a 11-gene expression signature that faithfully predicted the CDK4 modification profiles of breast tumors and cell lines (concordance rates of 84% and 100% in the 56 analyzed breast tumor samples or cell lines respectively). All three CDK4 modification profiles were evaluated in a merged independent dataset of 4034 published gene expression profiles. In these 4034 patients, 70% of triple-negative tumors, 18% of HER2-positive tumors and 5% of ER-positive tumors were predicted to have the first CDK4 profile wherein CDK4 phosphorylation is undetectable and to be completely unresponsive to CDK4 inhibitors. The phosphorylated CDK4 was predicted to be the major modified form in 26% of triple-negative tumors, 48% of HER2- positive tumors and 56% of ER-positive tumors. These patients should benefit the most from treatment with CDK4 inhibitors. Therefore, prediction of the CDK4 modification profile may allow extending treatment with Palbociclib to presently ineligible patients. As tumors with the third CDK4 modification profile generally present low grade and low OncotypeDX risks, the added value of including CDK4 inhibitors in their treatment compared to surgery and hormone therapy alone is questionable.

In conclusion, we identified CDK4 phosphorylation as the most direct biomarker of CDK4 inhibitor sensitivity in breast cancer and developed a promising 11-gene based surrogate marker to guide their use in the clinic.

Hypoxia, DNA methylation and retrotransposons: an opportunity for cancer immunotherapy?

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Radiotherapy-activated colorectal cancer-associated fibroblasts promote tumor progression through paracrine IGF-1R activation

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Title: Nanobody-mediated imaging of the immune checkpoint ligand PD-L1

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Abstract text:

Blockade of the PD-L1/PD1 signaling axis with antibodies specific for PD-L1 has shown clinical success in subsets of cancer patients. Moreover, radiolabeled PD-L1-specific antibodies have been explored for tumor stratification. However, antibodies have inherent limitations that can curtail their efficacy as theranostics.

We generated and characterized 37 nanobodies (Nbs) recognizing mouse PD-L1 and belonging to 13 sequence families. Among those, two Nbs C3 and E2 were selected based on in vitro and in vivo parameters and evaluated for imaging purposes. We performed SPECT/CT imaging in C57BL/6 wild type versus PD-L1 knock out mice, using Technetium-99m labeled Nbs. Nb C3 and E2 showed specific antigen binding and beneficial biodistribution. Next, we inoculated C57BL/6 wild type mice with lung epithelial TC-1 tumor cells or TC-1 cells modified with a lentiviral vector harboring shRNA specific for mouse PD-L1 (knock down). Delayed tumor growth was observed with cells showing low PD-L1 expression showing the central role of PD-L1 in regulating immune destruction. Upon SPECT/CT imaging with Nbs C3 and E2, we observed strong accumulation of the Nbs in tumors and showed that the Nb accumulation correlated with PD-L1 expression. Surprisingly, the highest PD-L1 expression and concomitant Nb accumulation was observed in tumors grown from PD-L1 knock down TC-1 cells. We next decided to generate PD-L1 knock out TC-1 cells using lentiviral vectors harboring CRISPR/Cas9 targeted to mouse PD-L1. Tumor growth after subcutaneous delivery of these cells in PD-L1 knock out mice could only be obtained in mice depleted of CD8 T cells. We verified that these tumors were devoid of PD-L1 expression, after which we performed SPECT/CT imaging using Nb C3 and E2. We showed that Nbs specifically only accumulated in PD-L1 expressing tumors, thereby confirming the diagnostic potential of the selected Nbs .

In conclusion, these data show that Nb C3 and E2 can be used to image PD-L1 in the tumor, and that the strength of the signal correlates with PD-L1 levels. These findings warrant further research into the use of Nbs as a tool to image inhibitory signals in the tumor environment.

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INHIBITION OF THE GLYCOLYTIC ACTIVATOR PFKFB3 IN ENDOTHELIUM INDUCES TUMOR VESSEL NORMALIZATION, IMPAIRS METASTASIS, AND IMPROVES CHEMOTHERAPY

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Endothelial cells line blood vessels to supply oxygen and nutrients to tissues. Abnormal vessel sprouting promotes cancer growth and spread. Traditional anti-angiogenic strategies attempt to reduce the tumor vascular supply by pruning tumor vessels, but their success is restricted by insufficient efficacy or development of resistance. An alternative strategy is to normalize the structurally and functionally abnormal tumor vessels, which would result in improved blood perfusion, decreased hypoxia (known to confer resistance to radio-, chemo-, and immune therapies) and increased drug accessibility. Starving endothelial cells in blood vessels from nutrients and energy through blocking their metabolism represents a novel strategy to inhibit angiogenesis. We recently showed that tumor endothelial cells have a hyper-glycolytic metabolism, necessary for their biomass production and proliferation. EC haplodeficiency or pharmacological blockade of the glycolytic activator PFKFB3 did not affect primary tumor growth, but reduced cancer cell invasion, intravasation and metastasis by normalizing tumor vessels, which improved vessel maturation and perfusion. Mechanistically, PFKFB3 inhibition tightened the vascular barrier by reducing VE-cadherin endocytosis in endothelial cells, and rendering pericytes more quiescent and adhesive (via upregulation of N-cadherin) through glycolysis reduction. PFKFB3 inhibition also impaired cancer cell extravasation by lowering the expression of cancer cell adhesion molecules in ECs via reduction of NF-κB signaling. These findings suggest that PFKFB3 blockade could represent a novel therapeutic tumor vessel normalization strategy.

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In vitro study on the potential of afatinib to overcome intrinsic and acquired cetuximab resistance in HNSCC cell lines

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