Cross-resistance between Cdk4/6 kinase inhibitors and castration in prostate cancer models

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Non-organ confined prostate cancer (PCa) is often effectively, but only transiently treated by targeting the androgen receptor (AR) signaling axis through androgen depletion strategies, often coupled with AR antagonists. Unfortunately, disease recurs within a median of 3-4 years, presenting as castration resistant PCa (CRPC), for which there are limited therapeutic options. This emphasizes the need for more efficacious drugs and a patient-tailored approach towards cancer therapy to improve disease outcome.

One class of drugs currently tested clinically, Cdk4/6 kinase inhibitors (e.g. palbociclib), blocks phosphorylation of the retinoblastoma (RB) tumor suppressor, thereby boosting its function, and likely preventing castration resistance. As Cdk4/6 inhibitor resistance has already been reported in other cancers, some PCa patients are anticipated to develop drug resistance. Here, we created palbociclib-resistant PCa cell models by continuously culturing them in presence of the drug to unravel mechanisms of acquired resistance, and assessed them for cross-resistance to ribociclib, hormone deprivation, and androgen signaling targeted therapeutics.

While in wildtype hormone therapy (HT) sensitive cells, palbociclib or ribociclib treatment efficiently induces a cell cycle arrest in G1, the palbociclib-resistant cell lines bypass this. Although loss of RB is a known mechanism for Cdk4/6i resistance, none of the models lost RB expression. Palbociclib and ribociclib do not block phosphorylation of RB in the resistant models, suggesting reactivation or bypass of this kinase pathway via rewiring of the kinome, evidenced by altered total phospo-Tyrosine and –Serine immunoblotting. A shotgun phosphoproteomics approach will identify specific kinases and proteins that are differentially phosphorylated (TBD). *In vivo*, tumor take of palbociclib-resistant PCa cells is significantly faster (median: 19.5 days), compared to wildtype (28 days), suggestive of a more aggressive phenotype.

Strikingly, these originally HT-sensitive cell lines, upon developing palbociclib resistance, acquired cross-resistance to androgen deprivation, as well as an AR antagonist (enzalutamide). RNA sequencing identifies downregulation of AR signaling, while AR protein is retained, suggesting that these cells do not rely on reactivation of AR for their enhanced proliferation. Our resistance models demonstrate an enriched GSEA EMT signature, currently being validated via migration/invasion assays.

Taken together, the enhanced proliferation, putative invasiveness, and independence of AR would suggest a more aggressive form of disease when palbociclib resistance occurs. Our data imply that patients who develop Cdk4/6 inhibitor resistance are likely to be castration resistant, and therefore would not benefit from AR signaling based therapeutics, such as the standard of care therapeutics enzalutamide in advanced, recurrent prostate cancer, and other strategies would need to be considered for patients that progress on CDK4/6 kinase inhibitors.

Conflict of Interest

N/A

Funding

This work is supported by grants from NIH (RO1 CA099996) and Novartis.

RdL is supported for this project by a Prostate Cancer Foundation Young Investigator Award