Inactivation of Aurora kinases and Cyclin-dependent kinases 4/6 allows cancers to adopt an endoreplication and form polyploid/polyaneuploid giant cancer cells (PGCCs/PACCs) that resist antimitotic drugs

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Abstract

Polyploid/Polyaneuploid giant cancer cells (PGCCs/PACCs) are common in tumors and have been tightly linked with tumor heterogeneity, resistance to cancer therapy, tumor relapse, metastasis, malignancy, immunosuppression, modulation of the tumor microenvironment and cancer stem cells. The abundance of PGCCs/PACCs is markedly higher in high-grade malignant tumors than in low-grade tumors, in the metastatic foci than in the primary tumor, and in relapsing tumors post-chemotherapy than in tumors before therapy. Immunosuppressive proteins including programmed death-ligand 1 (PD-L1) were also found to be overexpressed in these cells. Such cells are known to escape from cytotoxicity induced by major anti-cancer agents including taxanes, vinca alkaloids and platinum-based chemotherapies. Therefore, they are responsible of contributing to a microenvironment advantageous for tumor growth and survival. However, the molecular mechanisms that cause these cells to form were not yet known.

PGCCs/PACCs can repopulate *in vitro* as they generate tumors when inoculated into mice. Their daughter cells acquire a mesenchymal phenotype, which is a key transformation for cancer development, progression and metastasis. Emerging evidence has demonstrated PGCCs/PACCs arise in lung cancer, cervical carcinoma, ovarian cancer, prostate cancer, glioblastoma, colorectal cancer and breast cancer. Therefore, revealing the molecular events that cause PGCCs/PACCs to form could lead to clinically relevant approaches for treating recurrent and metastatic disease.

With our studies, we discovered that Aurora kinases and Cyclin-dependent kinases 4/6 (CDK4/6) were two separate synergistic determinants of distinct switches from the proliferative cell cycle to polyploid growth state in lung cancer cell lines. When Aurora kinases are inhibited together, cancer cells uniformly grow into multinucleated PGCCs/PACCs whereas inactivation of CDK4/6 forms mononucleated PGCCs/PACCs. These cells adopt an endoreplication in which the genome replicates, mitosis is omitted and cells grow in size. Consequently, such cells continue to safely grow in the presence of anti-cancer agents. These PGCCs can reenter the proliferative cell cycle and grow in cell number when the treatment is terminated.

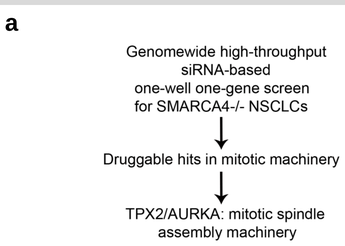
Based upon our results, we were funded to find chemical inhibitors to target PGCCs/PACCs. We have conducted a high-throughput screen of 332,500 chemicals of the UT Southwestern chemical library to identify those that were toxic to our representative cell line model, but not to cells with normal ploidy, including immortalized normal human bronchial epithelial cells (HBECs). Currently, in our research program, we have two major projects, which includes: **(1)** A complete biological and mechanistic characterization of PGCCs/PACCs in cancer initiation, progression, metastasis and drug resistance, and **(2)** development of inhibitors to target PGCCs/PACCs as drug candidates for cancer therapies.

To the best of our knowledge, ours are the first studies to describe the responsible genes involved in the formation of PGCCs/PACCs.

Background

Figure 1. *We performed a genome-wide high-throughput siRNA-based screening to identify genes required for survival of NSCLCs harboring SMARCA4-inactivating mutations.*

a



Tagal et. al, Nat. Commun. 2017. 8, 14098.

Background

Figure 2. *Genome-wide high-throughput siRNA-based screening identified Aurora kinase A potentially required for the survival and growth of NSCLCs harboring SMARCA4-inactivating mutations.*

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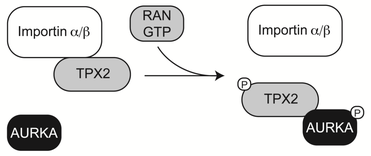
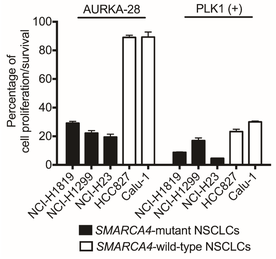


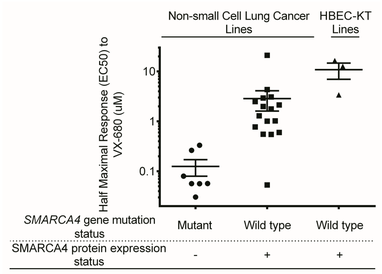
b *Depletion of Aurora kinase A caused cytotoxicity in SMARCA4-inactivated NSCLCs.*



c *Response of NCI-H1819 cells to AURKA inhibitors.*

Drug	EC50 (uM)
VX-680	0.045
MLN8237	0.041
PHA-739358	0.105
ENMD-2076	0.569
VX-689	0.694

d *SMARCA4-mutant NSCLCs were hypersensitive to Aurora kinase inhibitor, VX-680.*

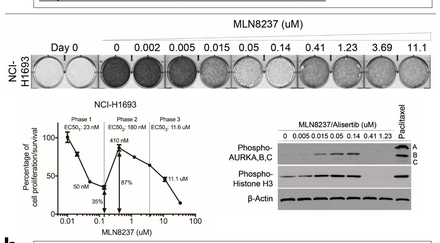


Tagal et. al, Nat. Commun. 2017. 8, 14098.

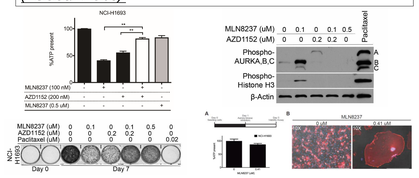
Results

Figure 3. *Inactivation of three Aurora kinases together produced more polyploid/polyaneuploid cancer giant cells (PGCCs/PACCs) than inactivation of Aurora kinase A alone.*

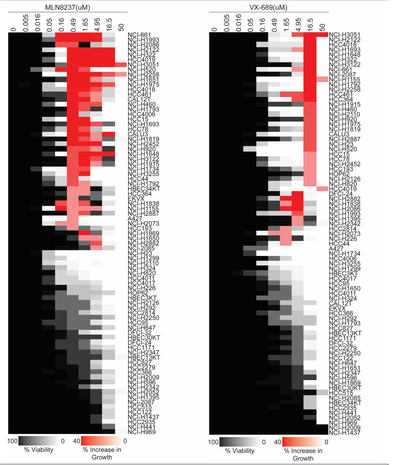
a *Multiphasic drug response curve pattern of NCI-H1693 cells in response to Aurora kinase A inhibitor, MLN8237.*



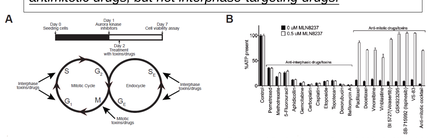
b *Co-inhibition of Aurora A with MLN8237 and Aurora B with AZD1152 in NCI-H1693 cells resulted in more cancer persister cells in the form of polyploid/polyaneuploid giant cancer cells (PGCCs/PACCs).*



c *Survey of NSCLC lines revealed that 60% of cell lines had more PGCCs/PACCs at higher concentrations of AURKi in comparison to lower concentrations. Red indicates increased cell growth.*



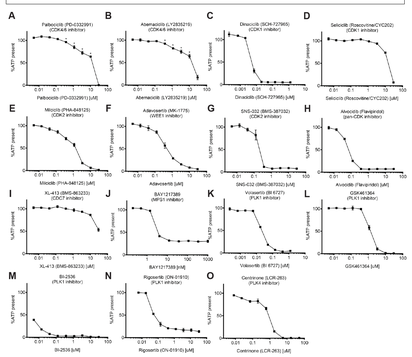
d *Inhibition of Aurora kinase signaling provided resistance to antimitotic drugs, but not interphase-targeting drugs.*



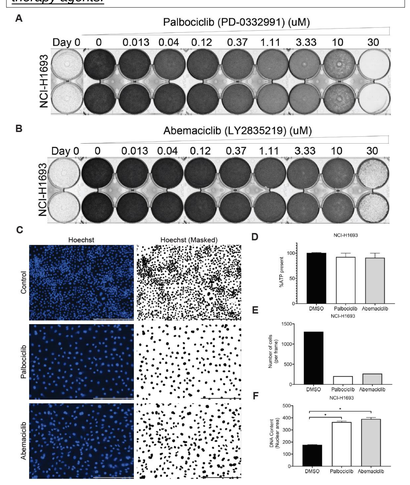
Tagal and Roth, Cancer Res.. 2021. 81(2), 4001-413.

Figure 4. *Inactivation of Cyclin-dependent kinase 4/6 (CDK4/6) signaling allows cancer cells to develop into PGCCs/PACCs and resist anticancer drugs.*

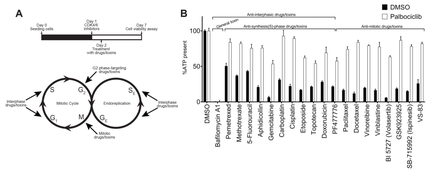
a *Screen of 15 different new generation cell cycle inhibitors targeting various kinases and phosphatases revealed that loss of Cyclin-dependent kinases 4/6 signaling produced PGCCs/PACCs. * indicates the concentrations of inhibitors that PGCCs/PACCs were observed.*



b *Mononucleated PGCCs/PACCs formed with CDK4/6-targeted therapy agents.*



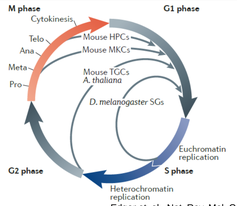
c *Inhibition of Cyclin-dependent kinases 4/6 signaling provided resistance to both antimitotic and interphase-targeting drugs.*



Unpublished Data

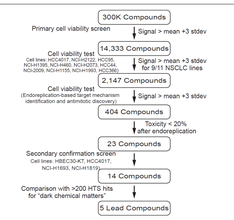
Figure 5. *Proposed model. Cells resistant to Aurora kinase and Cyclin-dependent kinase 4/6-targeted therapy adopt an endoreplication variant, where cell division is omitted, but genome replication and cell growth are preserved.*

a *Endoreplication mechanisms exist in mammalian systems as well as in plants and many other animals to generate specialized cell types.*



Edgar et. al, Nat. Rev. Mol. Cell Biol. 2014. 15, 197-210.

b *High-throughput compound library screen to identify chemicals cytotoxic to cancer persister PGCCs/PACCs.*



Unpublished Data

Conclusions

- Aurora kinases and Cyclin-dependent kinases 4/6 are synergistic determinants of a switch from the proliferative cycle to polyploid growth in lung cancer cells.
- Loss of Aurora kinase or CDK4/6 signaling allows lung cancer cells to grow into multinucleated or mononucleated polyploid giant cancer cells (PGCCs), respectively.
- AURKi or CDK4/6i-induced PGCCs adopt an endoreplication in which the genome replicates, mitosis is omitted and cells continue to grow in size.
- Due to the loss of a need for the proliferative cell cycle machineries, such cells continue to safely grow in the presence of most anticancer agents.
- These PGCCs can re-enter the proliferative cell cycle and grow in cell number when the treatment is terminated.

References

1. Tagal V, Wei S, Zhang W, Brekken RA, Posner BA, Peyton M, Girard L, Hwang T, Wheeler DA, Minna JD, White MA, Gazdar AF, Roth MG. SMARCA4-inactivating mutations increase sensitivity to Aurora kinase A inhibitor VX-680 in non-small cell lung cancers. Nat Commun. 2017; 8: 14098.
2. Tagal V, Roth MG. Loss of Aurora kinase signaling allows lung cancer cells to adopt endoreplication and form polyploid giant cancer cells that resist antimitotic drugs. Cancer Res. 2021; 81(2):400-413.
3. Edgar BA, Zielke N, Gutierrez C. Endocycles: a recurrent evolutionary innovation for post-mitotic cell growth. Nat Rev Mol Cell Biol. 2014; 15 (3):197-210.

For further information

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