Developing an IF panel to examine Cyclin and CDK interactions in a Pancreatic Adenocarcinoma Patient Derived Xenograph.

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Background

Cyclins and cyclin-dependent kinases are known for controlling cell-cycle progression through various phases of the cell division cycle. Cyclins coordinate cell division by binding to their associated cdks and maintain genomic integrity by tightly controlling these processes. In cancer, cellular proliferation becomes dysregulated, implicating both cyclins and cdks. Complexes of therapeutic interest are Cyclin E1 (CCNE1) and CDK2, complexes that overlap with late G1/S phase and Cyclin A2 (CCNA2) and CDK1/2 that are essential for S-phase progression.

Objectives

• Validate specific Cyclin A2 antibody
• Develop a multiplex Cyclin-CDK immunofluorescent panel.
• Observe how Cyclins (CCNA2, CCNE1) interact with cyclin-dependent kinases (CDK2) in pancreatic adenocarcinoma patient derived xenograft.

Materials and Methods

siRNA experiments:
• CCNA2 and Non-targeting controls (NTC) siRNAs were acquired from Dharmacon.
• siRNA transfections were performed using DharmaFect formulation to optimize seeding density for 10 cm plates.
• To induce Cyclin A2 protein, HCT116 (human colorectal carcinoma cell lines) were treated with aphidicolin at 10uM for 16hrs and 30uM for 4 hours and released for 4 hours.
• Cells were collected 72 hours later for knock down validation using western blots and cytoblocks.
• HCT 116 cell line was used for generation of cytoblocks given its common availability and easiness to transfect.

Western Blotting
• Cells were lysed and protein concentration was determined using BCA assay.
• CCNA2, Non-targeting controls, and treated lysates were loaded and run on ice at 60V for 15 minutes and 180V for 40 more minutes.
• Gel was transferred via iBlot system, incubated with antibodies overnight and developed using Li-COR secondaries and read with Li-COR reader (Odyssey CLX).

IHC Optimization and 6-color multiplex CDK-Cyclin panel.
• Antibodies were first optimized by IHC using positive and negative controls, using serial 2-fold dilutions, then optimized in Opal IF using serial 4-fold dilutions.
• The multiplex panel was ordered based on the affinity and pattern of each stain, using a serial OPAL protocol.
• This process was repeated to include CycE1 and CDK2, CDK1 and HLA (Human Leukocyte Antigen) were developed using anti-mouse and anti-rabbit fluorescent secondaries, respectively.

Results

Figure 2. Immunoblotting of siRNA CCNA2 knock-down efficiency with different cell densities. KD1 was used to create cytoblocks for further IHC validation.

Figure 3. Immunohistochemistry of HCT116 cells probed Cyclin A2. (A) shows a Non-targeting control (wild-type, low positive expression) (B) is siCCNA2A (negative expression) (C) and (D) show treated HCT116 aphidicolin cells with 10uM and 30 uM respectively (over-expression).

Figure 4. Confirmed optimization of PDAC PDX model is shown by both low and high CCNA2 expression.

Figure 5. Dilution optimization of Opal simplex in PDAC PDX models.

Figure 6. Multiplex panel PATX PDX model with CDK2, CycE1, CycA2, CDK1, and HLA biomarker detection.

Conclusions

• Multiplex panel of Cyclins and CDKs (complexes) was optimized and developed.
• Cyclin and CDK phenotypes were observed and quantified.
• Subcellular localization of each cyclin-cdk complex shows key differences in PDAC PDX.

References

1) Ballina Garcia-Reyes, Anna Laura Kretz, Jan-Philipp Ruff, Andreas Hiltbrand, Uwe Knippschild, Doris Henne-Brun, Johannes Lemke. (2018). The Emerging Role of Cyclin-Dependent Kinases (CDKs) in Pancreatic Ductal Adenocarcinoma. MPMI

Figure 1. Depicts the cell-cycle phases and their associated CDK/cyclin complexes (International Journal of Molecular Sciences, The Emerging Role of Cyclin-Dependent Kinases (CDKs) in Pancreatic Ductal Adenocarcinoma).