Characterization of KRas pathway inhibitors in 2D and 3D screening formats

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Introduction

The KRas/RAF/MEK signaling axis has been identified to play a critical role in the formation of cancer, resulting in several investigational new drugs targeting this pathway.1

Identification of appropriate drugs is hampered, however, by the assumption that a three-dimensional setting may be required to observe significant inhibitory effects, possibly due to alternate gene expression and protein activity in such a “closer-to-physiology” setting. In order to explore that topic, in the current study we have analyzed the activity of above indicated inhibitors in our 140 cell lines (CL-2D proliferation assay (3 days incubation)) and our 100CL-3D Soft Agar panel (> 7 days incubation). Selected settings have been tested in a 3D spheroid assay. Comparative analysis of overlapping cell lines of both panels clearly supports the notion that potencies tend to be higher in the 3D situation when targeting this pathway.

Focus on cell lines MiaPaCa2, HCC827 and A549

Analysis of exceptionally sensitive cell lines of each KRas inhibitor target group, i.e. KRasG12C mutated MiaPaCa2 pancreatic tumor cell2 or EGFR-mutated cell lines mutated HCC827 NSCLC tumor cells as target for 3D spheroids revealed pronounced susceptibility of these cell lines in 3D only to the respective inhibitor group, but not to the other seven spheroids. In contrast to literature, however, significant values of SOS inhibitors on KRasG12C mutated A549 NSCLC tumor cells were reproducibly and observed. We therefore explored these cells in a nother 3D system shown in the next figure.

Comparison of the IC50 profile of KRas pathway inhibitors in 3D Soft Agar growth vs 2D proliferation

Cells from the 100 CL 3D Soft Agar and the 140 CL 2D proliferation panel were tested for their response to indicated KRas pathway inhibitors. For the 86 cell lines overlapping within both panels, the potency of each panel was plotted to compare data generated under both conditions. Results showed that inhibitors of different extent act more potently in 3D than in 2D, which was specifically pronounced with the RAF and MEK inhibitor. No significant potency difference was observed e.g. with proteasome inhibitor Bortezomib (data not shown). Cell lines indicated with an arrow were chosen for a close-up look in the next figure.

Method

Principle of the 3D and 3D spheroids assay:

2D proliferation assay: Cells were seeded in white clear-bottom 384-well plates. Next day, compounds were added using a nanoliter-dispenser (Tecan, MSL). After incubation for 3 days, cell viability was measured using CellTiter-Glo (Promega). 

3D spheroid assay: Cells were seeded in clear round-bottom ultra-low attachment 384-well plates. After plating, 3D-spheroids with 50-60% confluence were added using a nanoliter-dispenser (Tecan, MSL). After 7 days incubation, spheroids were stained and then loaded with 3C-12C before transfer to white plates for luminescence measurement.

3D Soft Agar assay: Cells were seeded in 500-μl Soft Agar in clear 384-well plates (100 μl Soft Agar on day 1, compounds were added using a nanoliter-dispenser (Tecan, MSL). After 7 days incubation, spheroids were stained and then loaded with 3C-12C before transfer to white plates for luminescence measurement.

2D proliferation assay:

3D spheroid assay:

3D Soft Agar assay:

Summar y

Comparison of potencies for six KRas pathway inhibitors on 86 cell lines in 3D versus 2D cellular assays showed that:

- Overall, a higher potency is observed in 3D.
- This broadly observed with RAF- and MEK kinase inhibitors
- In many cases, compounds indicate a trend of activity in 2D, which is now pronounced in 3D (e.g. Sotarab or Selumetinib on MiaPaCa2 cells).
- In some cases, compounds appear inactive in 2D, but very potent in 3D (e.g. B3406 and Selumetinib on HCC827 cells).
- Especially for moderate effects, testing different 3D assays may be of use to identify adequate inhibitors (e.g. B3406 on A549 in Sphe roids vs Soft Agar assay).
- Our results show that 3D growth analysis clearly supports the development of KRas/Raf/Mek pathway inhibitors.

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References


Fig. 1: IC50 distribution in 2D Soft Agar vs. 2D proliferation over the whole panel of cell lines. Obtained IC50 values for the 86 cell lines were sorted from highest to lowest based on the IC50 reached which are depicted as red line. The corresponding values for the 2D proliferation assay are shown as black line. The plot shows that the IC50 line is generally below the black 2D proliferation line, supporting the notion that KRas pathway inhibitors show better activity in a three-dimensional setting.

Fig. 2: Dose-response curves in overlay plots of the six inhibitors on indicated cell lines. Raw luminescence data were converted into percent cell viability relative to the high and low controls, which were set to 100% and 0%, respectively. IC50 was calculated with GraphPad software and a variable slope dose response fitting model using the parameter as bottom constant and 100% viability as top constant. Shown are means ± SD. Data obtained from 3D Soft Agar and 2D Proliferation experiments are shown in red and black, respectively.