Inhibition of BCKDK to increase the sensitivity of breast cancer cells to paclitaxel

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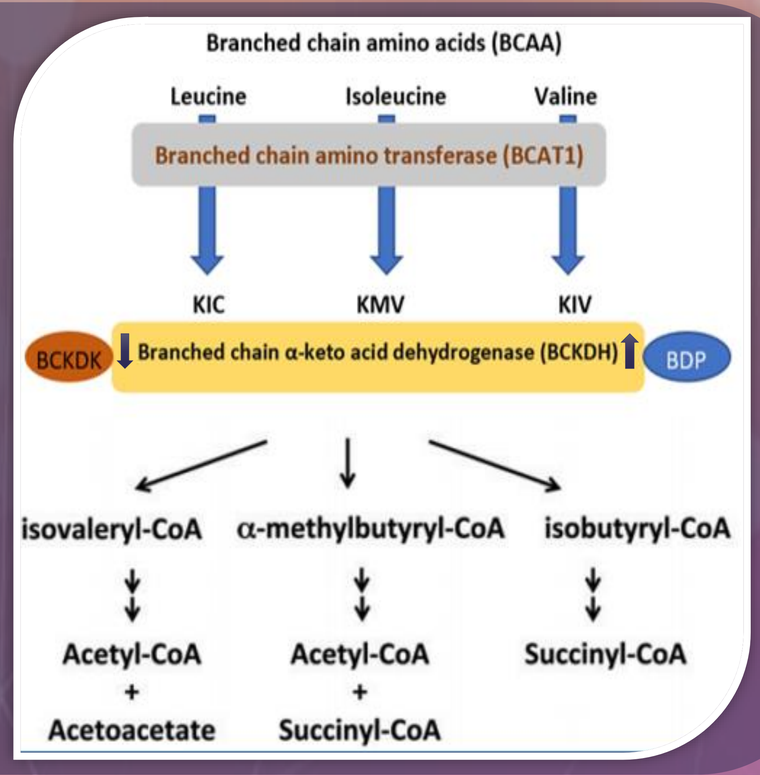
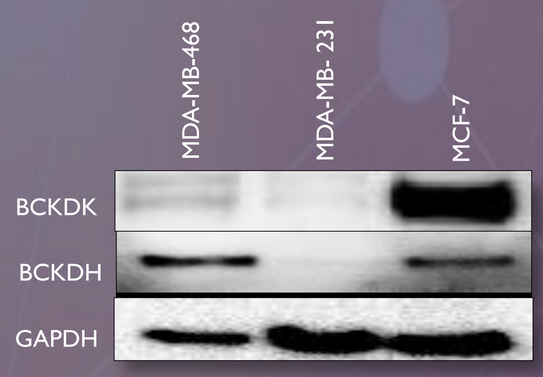
BACKGROUND

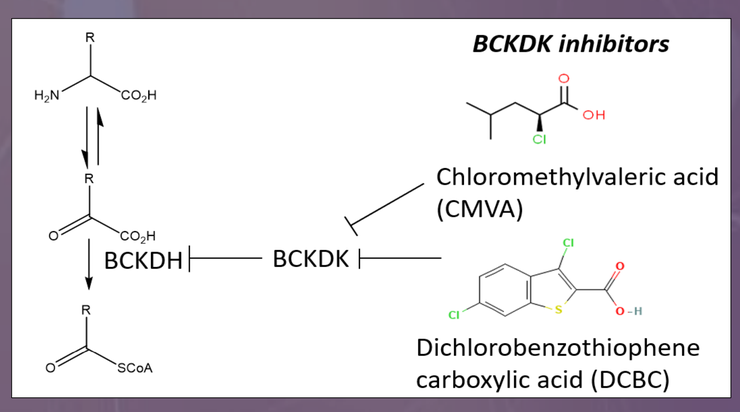
- Breast cancer is the most diagnosed cancer among women worldwide. Despite the fact that the management of breast cancer continues to improve, breast cancer still constitutes one of the most common and serious cancer types in females. A significant challenge in the management of breast cancer is that the number of drug resistant cases remains high (Cosentino et al., 2021).
- Our lab group had previously established an RNAi-based screen to identify genes that could contribute to drug resistance. One of the genes implicated in resistance to paclitaxel was the branched-chain keto-acid dehydrogenase kinase (BCKDK,Vidot et al., 2010).
- BCKDK is a key regulator of BCAA metabolism that inhibits the branched-chain keto-acid dehydrogenase (BCKDH).
- Branched-chain amino acids (BCAAs) are essential amino acids for humans and play roles in the protein synthesis and turnover, cell signaling pathways, glucose metabolism, synthesis of nitrogenous compounds, and signaling molecules, intestinal health, and immunity. BCAA regulate the activity of mTORC1 (Nie et al., 2018).



BCKDK and BCKDH levels in different breast cancer cell line

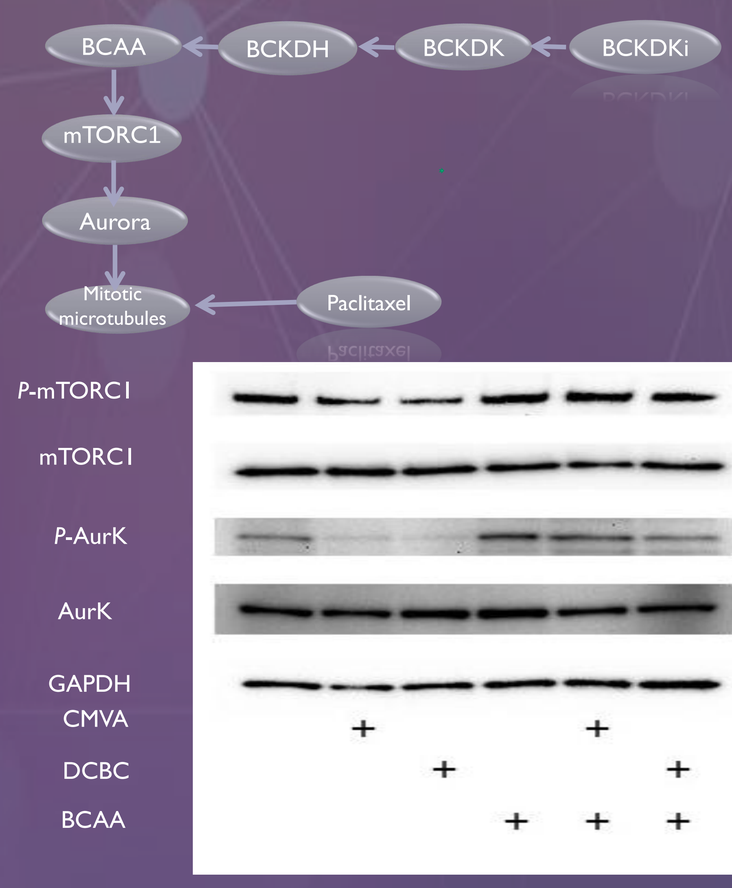


BCKDK INHIBITORS (BCKDKi)



- Previously, we have found BCKDKi are synergistic with paclitaxel and that this is likely to be due to inhibition of the mTORC/Aurora pathway
- Here we have confirmed and further characterized the mechanism underly the synergy

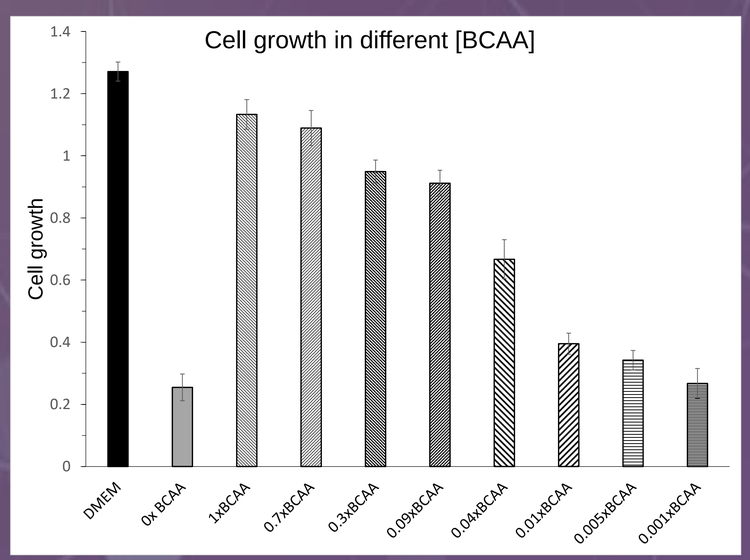
Proposed model explaining synergy



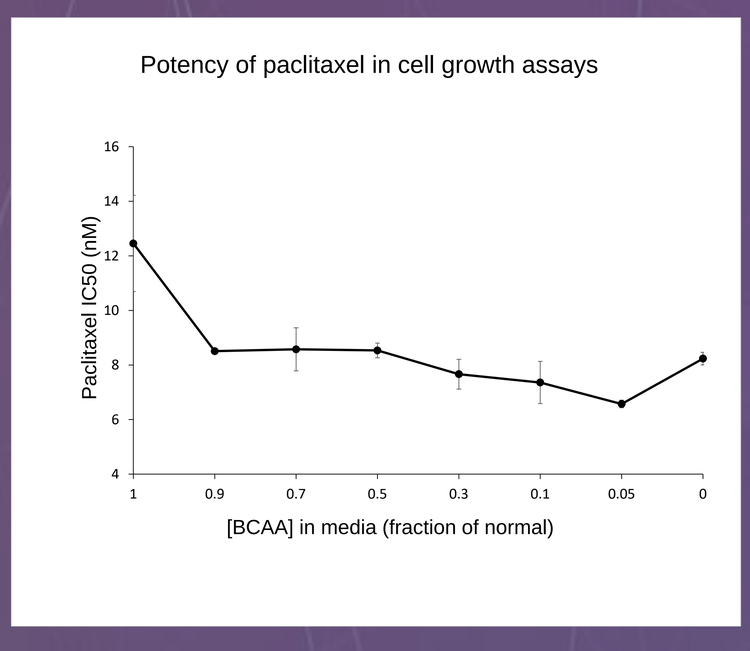
- BCKDK inhibitors regulate the BCAA-mTORC1-AurK axis.
- BCAA are known to regulate mTORC1, which in turn regulates Aurora kinase (AurK). Considering that AurK regulates mitotic microtubules, the target of paclitaxel, this provides a likely basis for the synergy we observed. BCKDKi were found to inhibit mTORC and AurK, as well as levels of Myc which is also regulated by mTORC and AurK.

RESULTS

Considering that BCKDKi reduce intracellular [BCAA], to mimic this we prepared bespoke cell culture media with varying [BCAA] to explore the effect on sensitivity to paclitaxel



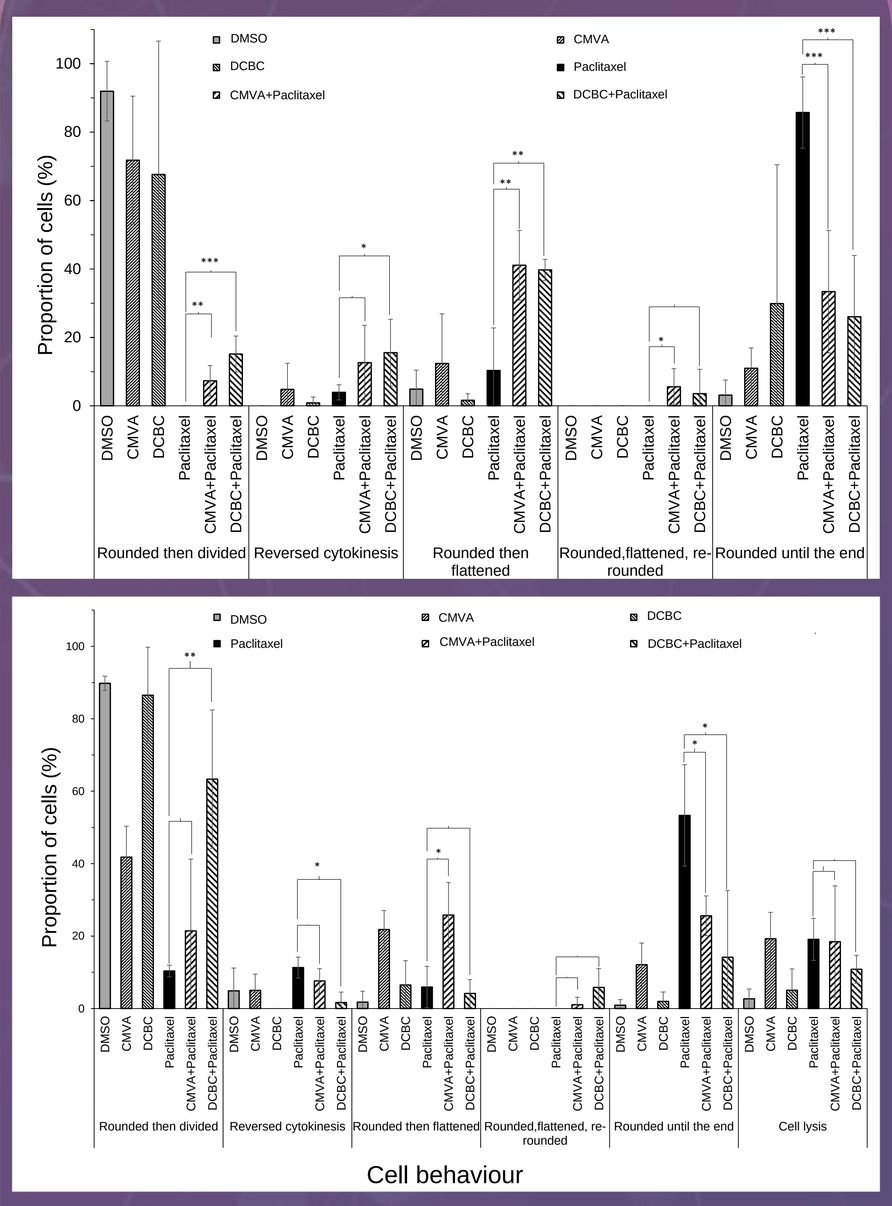
Potency of paclitaxel in cell growth assay



- The IC_{50} of paclitaxel decreased in media with reduced [BCAA].

Effect of CMVA, DCBC, Paclitaxel and their combination on cell behavior

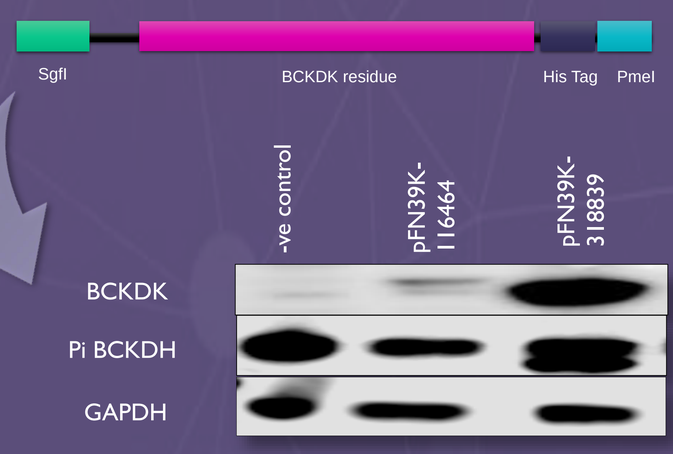
- Aurora A and B regulate several stages of mitosis, and the combination of BCKDKi and paclitaxel induced multinuclear cells.
- We examined the effect of the drug combination on cell division by video microscopy.



- Paclitaxel causes M phase arrest and cell rounding.
- CMVA and DCBC allowed the cell to escape paclitaxel-induced arrest and they either divided (mitotic slippage) or they apparently aborted mitosis and flattened.

Ectopic expression of BCKDK

- pCMV6 BCKDK clones 116464 and 318839 (ORIGENE), were PCR amplified to insert the Flexi blend enzymes sites and a His tag then ligated into pFN39K and expressed in HEK293 cells.



- Western blot analysis of BCKDK, P_i-BCKDH and GAPDH after transfection of BCKDK clones.

CONCLUSION

- BCKDK represents a novel target for sensitizing breast cancer cells to paclitaxel.

UPCOMING PLAN

- Ectopically express BCKDK to establish an enzyme assay to characterize novel BCKDKi

REFERENCES

1. Cosentino, G. et al. (2021) 'Breast Cancer Drug Resistance: Overcoming the Challenge by Capitalizing on MicroRNA and Tumor Microenvironment Interplay', Cancers 2021, Vol. 13, Page 3691, 13(15), p. 3691. doi: 10.3390/CANCERS13153691.
2. Vidot, S. et al. (2010) 'Autotaxin delays apoptosis induced by carboplatin in ovarian cancer cells', Cellular signalling, 22(6), pp. 926–935. doi: 10.1016/J.CELLSIG.2010.01.017.b
3. Nie, C. et al. (2018) 'Branched Chain Amino Acids: Beyond Nutrition Metabolism', International Journal of Molecular Sciences, 19(4), p. 954. doi: 10.3390/ijms19040954.