High Throughput screening for modulators of tissue-kallikrein expression

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ABSTRACT

Human tissue kallikreins (KLKs) comprise a subgroup of 15 homologous secreted serine proteases. Primarily known for their clinical use as cancer biomarkers (e.g. PSA), KLKs are found to have therapeutic value. A cell-based high throughput screening of 3 compound libraries (~4000 small molecules) was performed under fully automated robotic facilities and sensitive immunocassays (ELISA) were used to measure KLKs in a panel of different cell lines. The ‘validated leads’ were further assayed in-vitro, to measure their effectiveness (IC50s) at protein (ELISA) and mRNA levels (RT-PCR), cytotoxicity (LDH) and cell viability (MTT assay).

RESULTS

A) Initial screening

- Initial screening resulted in 66 ‘putative hits’ that decreased KLK expression by at least 50% over control.
- Secondary screening and mini-dose-response assays reduced this number to 21 ‘leads’.

Fig. 1. Initial Screening of the 3 libraries (~4000 compounds) identified 66 small molecules as putative hits. Those were further analyzed in the ‘secondary-validation’ step.

B) Mini-dose-response treatments with the ‘putative hits’ separated the ‘real leads’ from the false positives. A total of 21 lead-compounds were identified and proceeded to the IC50-calculation step.

Fig. 2. Mini-dose-response treatments with the ‘putative hits’ separated the ‘real leads’ from the false positives.

Fig. 3. Mini-dose responses with the 21 lead-compounds (A) and their relative cytotoxicity (LDH measurement).

C) Functional groups

- Ion pump inhibitors (cardiac glycosides)
- Translation inhibitors
- Trafficking inhibitors

Fig. 4. Classification of the 21 compounds. According to their mode of function these compounds can be grouped into three categories.

D) CARBAMIC ACID-CO2X (CBA)

Fig. 5. Sticking differences in the potency of compounds despite their very high structural similarities (same family).

E) Semi-quantitative RT-PCRs show a similar decrease in mRNA levels (in non-cytotoxic & non-apoptotic concentrations of the compounds).

CONCLUSION

The HTS worked very efficiently for our assay. Out of a total of 4000 compounds, 21 molecules (~0.5%) are validated as inhibitors of KLKs (5,6 and 10), which belong to 3 functional-families of compounds. Furthermore there was a very small overlap among the 3 libraries and these molecules came up as hits from all different libraries, reflecting the reproducibility & sensitivity of the technique.

RT-PCR experiments of treated cells show that these molecules block a certain pathway which regulates the expression of some proteins, including KLKs (data in progress). Further experiments to elucidate their exact mode of function are ongoing. This HTS-technique proved effective in identifying inhibitors of expression of our target protein throughout large chemical libraries. The precise pathways that seem to be blocked by these inhibitors and the therapeutic applications of their use, are currently under investigation.

REFERENCES


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