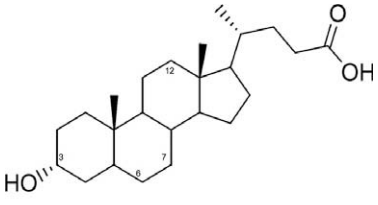
Materials and Methods: In order to find ligands for EphA2–ephrinA1 interaction we have set and performed an ELISA screening assay testing several natural and synthetic bioactive compounds.
Results: Binding studies identified lithocholic acid (LCA) as a competitive and reversible ligand of EphA2–ephrinA1 system in the order of 10 micromolar. Functional studies on HT29 colon cancer cells, naturally expressing EphA2, revealed that LCA dose-dependently antagonized EphA2 phosphorylation induced by ephrinA1-Fc.
In order to clarify the modality of interaction between LCA and EphA2 kinase we tested other naturally occurring bile acids and we synthesized a series of LCA derivatives exploring the positions 6,7,12 and the opposite ends of the molecule represented by the hydroxil group in position 3 and by the carboxyl moiety.
LCA derivatives resulted particularly sensitive to the modulation of the cyclopenta[a]perhydro phenanthrene scaffold. Indeed, the introduction of hydroxyl group in position 7 or 12, always produced inactive compounds, exemplified by the naturally occurring cholic, deoxycholic and chenodeoxycholic acid. Similarly, the introduction of a 6- or 7- keto group resulted detrimental for the binding affinity. The oxydation of the alpha hydroxyl group in position 3 as well as it acetylation led to compounds still able to interfere with EphA2–ephrinA1 system. Esterification or conjugation with taurine of the carboxylic group at the opposite end gave inactive compounds.
Conclusion: This study identified lithocholic acid as a competitive and reversible antagonist of EphA2 receptor. Moreover, it provided the basis for a rational chemical development of molecules targeting this new emerging system in the oncology field based on docking and molecular dynamics.



lithocholic acid (LCA)

294 POSTER

Identification of NPD3483 as a unique cell division inhibitor via the cell morphology-based screen

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Background: Recently, cell phenotypic profiling induced by chemical entities has been developed and recognized as an effective initial step in a small molecule probe- and drug-discovery. However, these approaches require many cellular profiling and specialized bioinfomatics techniques. The comprehensive analyses are too expensive, complicated and time-consuming to select a small number of "interesting" compounds from a large library. On the other hand, the tumor cellular shape undergoes often dynamic change specifically related to the mode of drug action. It prompted us to construct a simple cell morphology database, compiling various cellular phenotypes induced by authentic compounds, which may enable us to discover unique bioactive substances with approximately defined mechanism of action.
Material and Methods: We have examined the effect of 60 well-characterized drugs on cell-shape change in 3 mammalian cell lines (HeLa, tsNRK, and tsFT210 cells) in a time- and dose-dependent manner. Then we investigated the relationship between the induced morphological changes and the mode of action of a drug, allowing to easily discriminate the phenotypes induced by diverse types of drugs, such as cytoskeleton-interfering agents, and the inhibitors of HSP, HDAC and of macromolecular synthesis.
Results: We carried out the screening based on the morphological changes of cancer cells by using chemical libraries deposited in the RIKEN Natural Products Depository, NPDepo. In the course of the screening, we found the carboline derivative, NPD3483, inducing the cell-shape change similar to that of mitotic kinesin Eg5 inhibitors. This compound blocked cell division by affecting the formation of mitotic spindle; however the monoastral phenotype induced by well-known kinesin inhibitors was not observed. These data suggests that NPD3483 exerts its activity through a distinct mode of action from other Eg5 inhibitors.
Conclusions: To discover small drug like molecules, we constructed the cell morphology database, then explored chemical library and finally identified the NPD3483 as a unique cell division inhibitor. Our new screening system classifies well the mode of action of test compounds; moreover, it may provide a new insight into the regulatory mechanisms of the dividing cancer cells.

295 POSTER

High throughput screening of potential anti-cancer agents in primary cell culture using an ATP based luminescence assay

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Background: The development of anticancer agents commonly relies on screening potential drugs against cell lines. However, these show significant differences in their behaviour from tumor-derived cells in primary cell culture or xenografts. Nevertheless, cell lines continue to be used because of their convenience and utility for high throughput screens. We have developed a method which allows high throughput screening of anticancer agents in primary cell cultures.
Materials and Methods: In this study, 5605 plant extracts were each screened in 3 recurrent ovarian tumors. Cells were isolated from fresh tumor samples. These were incubated with 12.5μg/ml drug compound for 5 days in 384 well polypropylene plates (352 compounds per plate) before being lysed and ATP levels measured by luciferin-luciferase assay.
Results: There were 113 positive hits for sensitivity, where greater than 80% inhibition was achieved in all 3 tumor samples. These positive hits were then tested further for luciferase inhibition to rule out any compounds inhibiting the assay's detection system. Thirty-three of the 113 positive hits were excluded on this basis leaving 80 potential compounds to which these tumours were sensitive. Of the 80 hits obtained, 29 had previously been identified as active in a breast cancer cell line (ZR75) screen. Twenty showed toxicity against a fibroblast cell line (HS27) and were excluded from further investigation, leaving 60 extracts with activity of which 13 were previously positive in the breast cancer cell line screen.
Conclusions: This study shows a novel screening approach for large libraries of potential anti-cancer compounds which allows large numbers of agents to be tested against tumor-derived cells where previously the cell numbers required for such studies could not be achieved without use of cell lines. Of those agents selected, 47 were previously unknown from the cell line screen.

296 POSTER

Antitumor activity of NPD723, a novel potent cell differentiation-inducing agent in leukemic cells

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Introduction: Differentiation therapy is one of the promising strategies for treatment of leukemia. Here, we report the biological and antitumor activities of furocoumarin derivative NPD723, which is a newly identified cell differentiation-inducing compound from our chemical library, RIKEN Natural Products Depository (NPDepo).
Methods: Nitroblue tetrazolium reduction assay was used for the screening of cell differentiation-inducing compounds in HL-60 human leukemic cells. The induction of cell differentiation was evaluated by flow cytofluorimetry, RT-PCR, and western blot analyses. The anticancer activity *in vivo* was evaluated in mice xenografted with A549 human lung carcinoma. NPD723 was administered intraperitoneally at 10, 20, or 40 mg/kg on a weekly schedule of qdx5 for 2 weeks, and then tumor volume and body weight change were measured.
Results: Among 6,464 compounds tested, we found furocoumarin derivative NPD723 as the most potent compound. The activity of NPD723 was stronger than that of known differentiation-inducing agents, all-*trans*-retinoic acid (ATRA) and 1α,25-dihydroxy-vitamin D_3 (1α,25$(OH)_2D_3$); the EC_{50} values of NPD723, ATRA, and 1α,25$(OH)_2D_3$ were 0.8, 1.8, and 186.4 nM, respectively. Flow cytometric analysis showed that NPD723 induced the expression of CD11b and CD14, membrane markers of differentiation. NPD723, unlike ATRA or 1α,25$(OH)_2D_3$, did not induce the expression of retinoic acid receptor-β (RARβ) and vitamin D_3-24-hydroxylase, as determined by RT-PCR. These results suggest that NPD723 induces myeloid differentiation of HL-60 cells through a mechanism different from ATRA and 1α,25$(OH)_2D_3$. In addition, NPD723 strongly inhibited cell proliferation of various human cancer cell lines; IC_{50} values were 3.8 nM for HL-60 cells, 5.9 nM for A549 cells, and 9.3 nM for human prostate carcinoma DU145 cells. In the A549 xenograft model, NPD723 significantly inhibited tumor growth without significant loss of body weight.
Conclusions: NPD723 potently induced myeloid differentiation of HL-60 cells, and inhibited tumor growth *in vitro* and *in vivo*. These results suggest that NPD723 might be a novel anticancer agent active against leukemia and lung carcinoma.