Comprehensive molecular characterization of new anaplastic thyroid carcinoma cell lines reveals RhoB as a molecular target for therapy


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INTRODUCTION

Anaplastic thyroid carcinoma (ATC) is a highly aggressive carcinoma with a mortality rate near 100% and is in need of new therapeutic options. One critical component of drug discovery is the availability of well-characterized cell lines derived from patient tumors for identification of molecular mechanisms related to tumor biology and drug responsiveness. A recent report indicates that up to 42% of thyroid cancer cell lines, including ATC, are redundant or not of correct tissue origin. We report four new ATC cell lines and the methodologies used to characterize the originating tumor tissue and derive cell lines. To validate the integrity of our newly derived cell lines, we confirmed historically the original diagnosis of the ATC tumor tissues and demonstrated identical matching of the short tandem DNA repeats (STR) and mutational status to the tumor-derived cell lines. This is the first time that molecularly characterized thyroid cell lines are matched to the originating tumor tissues. In addition, we fully characterized the cell lines for proliferative growth, mRNA expression of seven thyroid markers, four oncogenes and array CGH to identify novel deletions and amplifications. Previously in other cell lines, we showed that RhoB is a key signaling node for the growth inhibition of ATC. Using our new cell lines, we have identified five classes of compounds (FTI-277, GGTI-286, lovastatin, romidepsin, UCN-01) that upregulate RhoB and inhibit cell proliferation in a dose-responsive fashion. In conclusion, we have developed four molecularly characterized cell lines that further implicate RhoB as a molecular target for therapy in genuine ATC cell lines.

RESULTS

Confirmation of thyroid origin

Figure 1. A H&E of patient anaplastic thyroid tissue with predominant subtype. B Phase contrast images of live human anaplastic thyroid carcinoma cell lines, cell lines derived from patient tissues. C STR sequences of both patient and cell lines confirm identities of derived cultures. Passage number of cell lines are as indicated. D RT-PCR analysis confirms de-differentiation in anaplastic cell lines with transcription of some thyroid genes implicating thyroid origin.

RhoB is a molecular target

Figure 4. A Real time PCR of RhoB mRNA upregulation in response to drug treatment after 24 hours. B Western analysis confirms RhoB expression upregulation. C Western of cells transfected with scrambled and RhoB shRNA illustrates that RhoB induction can be blocked by siRNA. D Proliferation assay to examine RhoB dependent effects. Cells were transfected with scrambled and RhoB shRNA for 24 hours followed by 3 day drug exposure.

CONCLUSIONS AND FUTURE DIRECTIONS

- STR analysis of both patient tissues and established cell lines is essential for verifying identity to avoid redundancy and misidentification.
- Four new ATC cell lines have been comprehensively characterized exhibiting unique profiles and phenotypes to be used for preclinical models.
- All ATC cell lines exhibit a dose-dependent growth inhibition and RhoB induction in response to HDAC inhibitor (Romidepsin), panhormone inhibitors (FTI-277, GGTI-286), HMG Co-A reductase inhibitor (lovastatin) and Chk1 inhibitor (UCN-01).
- Lovastatin and UCN-01 induce apoptosis in ATC in vitro
- As a signaling node, RhoB was silenced to demonstrate growth inhibition dependence in at least Romidepsin and FTI-277
- Future studies include verification of GGTI-286, lovastatin and UCN-01’s RhoB independence

Molecular characterization

Drug responsiveness

Figure 2. A Summary table of mutations, translocations, and uniquely altered genes. Neither of the ATC cell lines harbor the RET/PTC nor PAHX/RARR fusion as routinely seen in other thyroid carcinoma subtypes. B Western analysis of frequently over-expressed oncopgenic proteins in ATC that can lead to cell survival. C Cellular proliferation curve over seven days demonstrates proliferative potential of the cell lines. Passage numbers are as indicated.

Figure 3. A Representative graph of 4 day treatment with 5 different classes of drugs demonstrating dose dependent inhibition. B Western analysis of PARP and cleaved caspase 3 to check for apoptotic responsiveness after 48 hrs.