A Test to Quantify Notch Pathway Activity in T cell Acute Lymphoblastic Leukemia Patients

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BACKGROUND

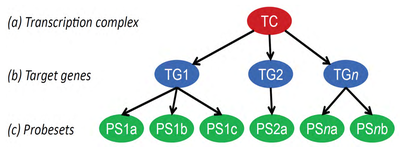
The Notch signaling pathway is pivotal for various physiological processes including immune responses, and has been implicated in the pathogenesis in many diseases including T-cell acute lymphoblastic leukemia (T-ALL). Over 70% of T-ALL patient samples contain mutations in NOTCH1 and/or FBXW7 that result in the activation of the Notch pathway. Various targeted drugs are available that inhibit NOTCH signaling, but their effectiveness varies due to variable Notch pathway activities among individual patients. Moreover, patients' leukemic cells that lack these mutations may still have tumor-driving NOTCH activity. A fast and robust quantification of Notch pathway activity in primary patient samples would identify patients who could benefit from NOTCH targeted treatment.

OBJECTIVES

In primary human T-ALL samples, we aimed to determine the Notch pathway activity in relation to active, intracellular NOTCH1 (ICN1) levels and in relation to NOTCH1 and/or FBXW7 mutations. Additionally, we investigated whether the Notch pathway activity score is more accurate than a mutation-based activity prediction.

MATERIALS & METHODS

The Notch pathway assay calculates a quantitative Notch pathway activity score from mRNA levels of conserved direct Notch target genes based on a Bayesian network model. This model describes the causal relation between up- or downregulation of NOTCH target genes and the presence of an active or inactive NOTCH transcription complex. Using this model, we scored Notch pathway activation in our well-characterized cohort of 117 T-ALL (treated as indicated) patient samples and related it to biological and clinical parameters including clinical outcome.



Bayesian model. Inference of transcriptional complex (TC) or pathway activity from the presence of target genes (TG) measured by the level of Affymetrix probesets (PS)

Selection of NOTCH target genes

Calibration: Affymetrix datasets malignant vs. healthy ovary

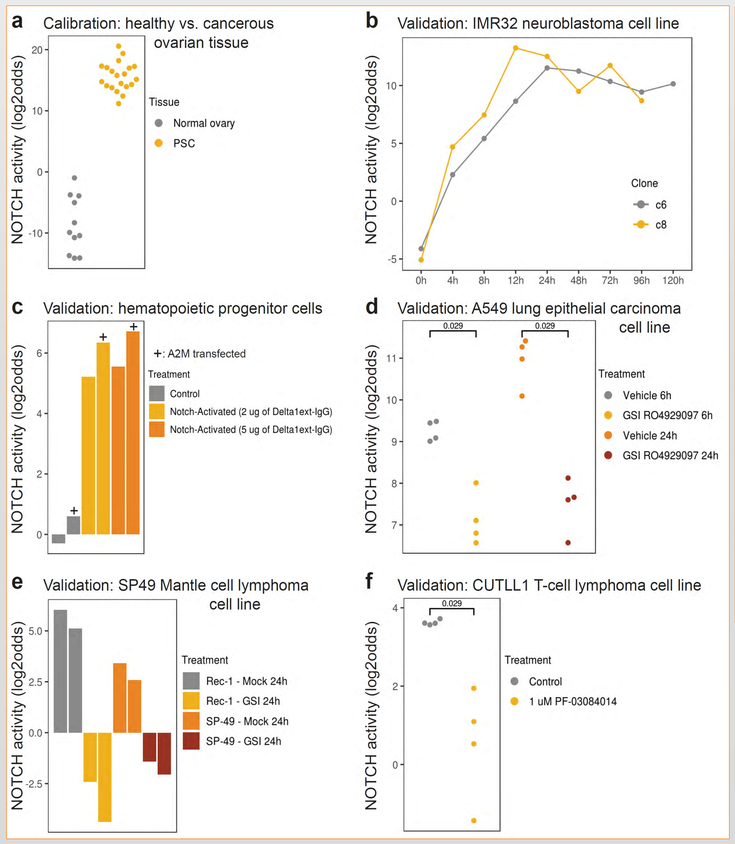
Validation: Affymetrix multiple public datasets, including diagnostic T-ALL

Notch pathway score calculation on the current cohort (n=117)

For research use only

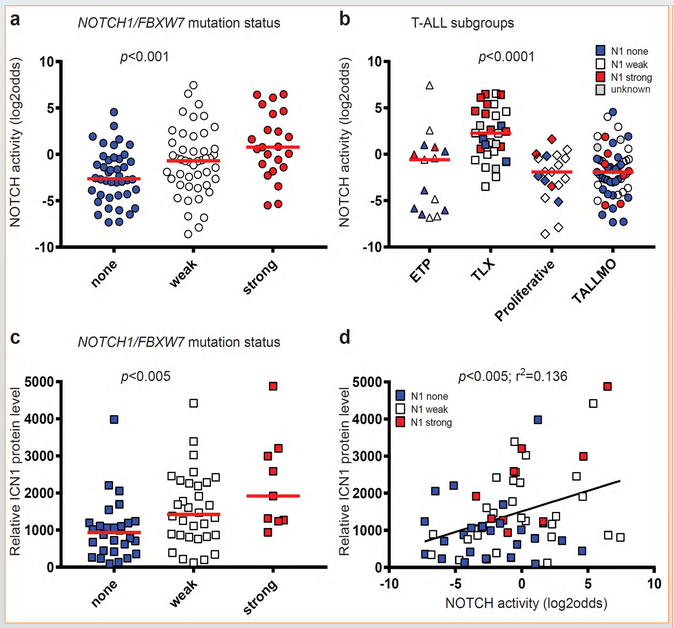
RESULTS

Notch pathway assay performs well in multiple cell types



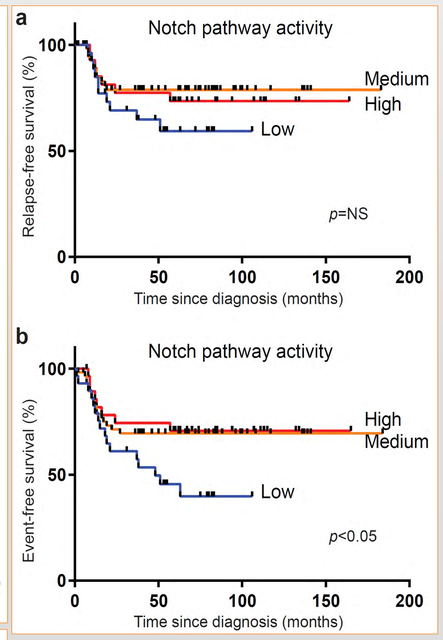
(A) Calibration using normal ovary (inactive pathway) and high grade serous ovarian cancer samples (active pathway). GSE7307, GSE18520, GSE29450, GSE36668, GSE2109, GSE9891. (B-G) Validation on independent GEO datasets from different cell lines. (B) Two clones (c6 and c8) of the IMR32 neuroblastoma cell line at different times (0-120 hours) after induction of active intracellular NOTCH3. GSE16477. (C) A2M (+ symbol) or control vector transfected CD34+CD45RA-Lin- hematopoietic progenitor cells from umbilical cord blood were cultured with NOTCH ligand Delta1ext-IgG. A2M is a nuclear-trapped mutant of AF1q/MLLT11. GSE29524. (D) A549 lung cancer cell line treated with gamma secretase inhibitor (GSI). GSE36176. (E) Rec-1 (containing an activating NOTCH1 mutation) and SP49 Mantle cell lymphoma cell lines treated with GSI. GSE34602. (F) CUTLL1 T-cell lymphoma cell line treated with GSI. GSE33562.

Notch pathway activity in T-ALL is associated with strong activating mutations and TLX subgroup



(A-D) No NOTCH activating mutations, weak NOTCH1 activating mutations (NOTCH1 heterodimerization domain, PEST domain or in FBXW7) and strong NOTCH1 activating mutations (juxtamembrane domain or more than one activating mutation). (A-C)Kruskal-Wallis statistical test. Median: red line. (D) Linear regression. Notch pathway activity of T-ALL samples per NOTCH1/FBXW7 mutation status group (A) and per T-ALL subgroup (B). (C) Active intracellular NOTCH1 (ICN1) protein level measured in relative intensity units using reverse-phase protein array (RPPA), indicated per NOTCH1/FBXW7 mutation status group. (D) Correlation of ICN1 protein level and Notch pathway activity score.

Notch pathway activity predicts survival of T-ALL patients



Kaplan Meijer. Three groups were separated based on the lowest 25% (blue), intermediate 50% (orange), and highest 25% Notch pathway activity (red). Relapse-free (A) and event-free (B) survival for T-ALL pediatric patients treated on DCOG ALL-7, -8, and -9 and COALL-97 protocols. NS: not significant.

REFERENCES

Pathway analysis references:
[1] Cancer Res. 2014;74(11):2936–45. [2] Oncotarget. 2014;5(14):5196–7.
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[5] Cancers. 2019;11(3):E293.

CONCLUSIONS

New Notch pathway activity test:

- Uses expression data of selected direct NOTCH target genes
- Applicable to all cell/cancer types
- Validated in T-ALL
- Translates to qPCR for easier implementation in diagnostics

- Notch pathway can be active in the absence of *NOTCH1/FBXW7* mutations
- High Notch pathway activity predicts survival in T-ALL patients treated on DCOG ALL-7, -8, and -9 and COALL-97 protocols
- Opens possibilities to explore the contribution of the Notch pathway activity in tumor formation and in the stratification of cancer patients
- Potential NOTCH targeted therapy on the basis of Notch pathway activity

Conflicts of interest: LH, HvO, WV, AvdS are employees of Philips Research. The other authors declare no conflicts of interest.