The tyrosine kinase NPM-ALK mediates malignant transformation of normal human CD4 T lymphocytes

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Purpose:
Anaplastic large-cell lymphoma (ALCL) is a childhood T lymphoma defined by the presence or absence of chromosomal translocation t(2;5)(p23;q35) leading to the ectopic expression of anaplastic lymphoma kinase (ALK), with nucleophosmin fusion being the most common. The NPM-ALK chimeric protein is constitutively activated and is highly oncogenic. Aberrant ALK activation triggers various prosurvival signaling pathways. ALK(+) ALCL cells resemble activated CD4 lymphocytes as, first, they harbor genomic rearrangements at the TCR (T-Cell Receptor) locus and secondly they strongly express the CD30 molecule (a cell surface receptor in the TNF-R superfamily). However, the repertoire of T-cell specific markers is often incomplete at the surface ALK(+) ALCL cells with for instance a very frequent loss of CD3. Part of these aberrant phenotypes observed in patients is due to the activity of NPM-ALK which mediates, via both STAT3 and DNA methyl transferase 1 (DNMT1), DNA hypermethylation and repression of several T cell genes. For ALK(+) ALCL, cell lines exists and they originate almost exclusively from clinically advanced tumors which precludes study of the early stages of carcinogenesis and the mechanisms of tumor progression. Transgenic mouse models developing NPM-ALK driven tumors exist but none of them truly recapitulate features of ALK(+) ALCL and are insufficient to conclude on the progression and the cell of origin of the disease.

Results:
We demonstrated that in vitro transduction of normal human CD4 T lymphocytes with a NPM-ALK-expressing lentivector results in their malignant transformation. Transformed cells become immortalized and display morphology and immunophenotype characteristic of patient-derived ALCL. These features are dependent on NPM-ALK and include perpetual cell growth, proliferation and survival. Analysis of DNA methylation by genome-wide DNA methylation strategy (Methyl-array/MethylationEPIC BeadChip, Illumina, Pôle technologique du CRCT) shows a global DNA hypomethylation associated with a local hypermethylation (Bisulfite pyrosequencing). Implantation of NPM-ALK transformed cells into immunodeficient mice results in formation of tumors indistinguishable from patients’ ALCL.

Conclusion:
Altogether these results suggest that NPM-ALK(+) generated cells are a good model to study the initial oncogene-host cell interactions and the early stages of carcinogenesis. Thus, they could be useful to decipher the mechanisms of malignant progression and to evaluate ALK inhibitors, which have already shown substantial efficacy in ALK-driven malignancies.