Molecular and clinical refinement of atypical neurofibroma

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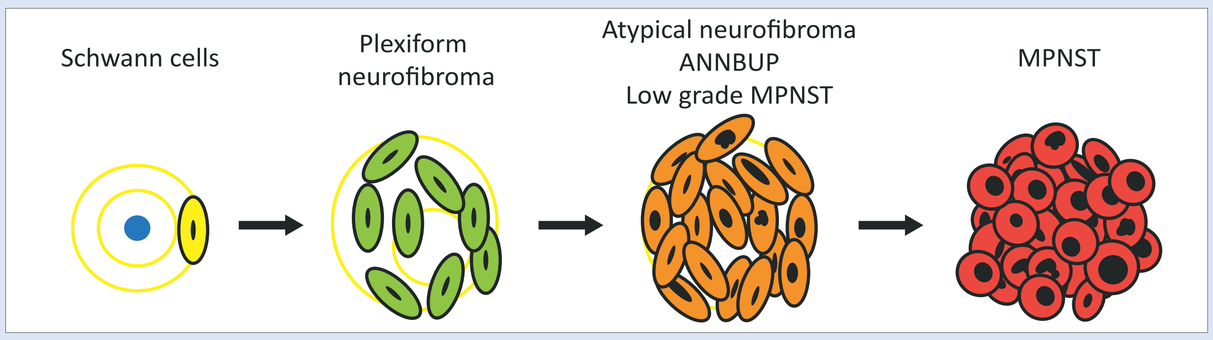
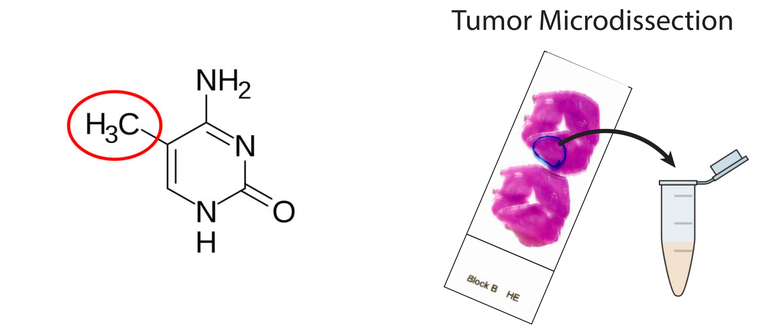
1. Introduction

Atypical neurofibroma (ANF) are at risk for progression to highly aggressive malignant peripheral nerve sheath tumors (MPNST). Therefore, the identification of such lesions is of high importance for risk adapted patient care and could help reduce the mortality of NF1 patients. Based on small series, different histological criteria have been proposed to identify "atypical neurofibroma" (ANF) or "atypical neurofibromatous neoplasms of uncertain biological potential" (ANNUBPs), but a satisfying consensus definition has not yet been reached. Most importantly, a thorough molecular and clinical characterization is missing. We aim to identify robust diagnostic markers for ANF and indicators for lesions at risk for malignant transformation.



2. Methods

Global DNA methylation profiling, which has emerged as a powerful tool for the classification of nervous system tumors, was performed in a series of 42 histologically defined ANF and integrated with clinical data. Data from 41 MPNST, 11 plexiform neurofibromas, 14 dermal neurofibromas, 68 schwannomas and 10 melanotic schwannomas served for comparative purposes.



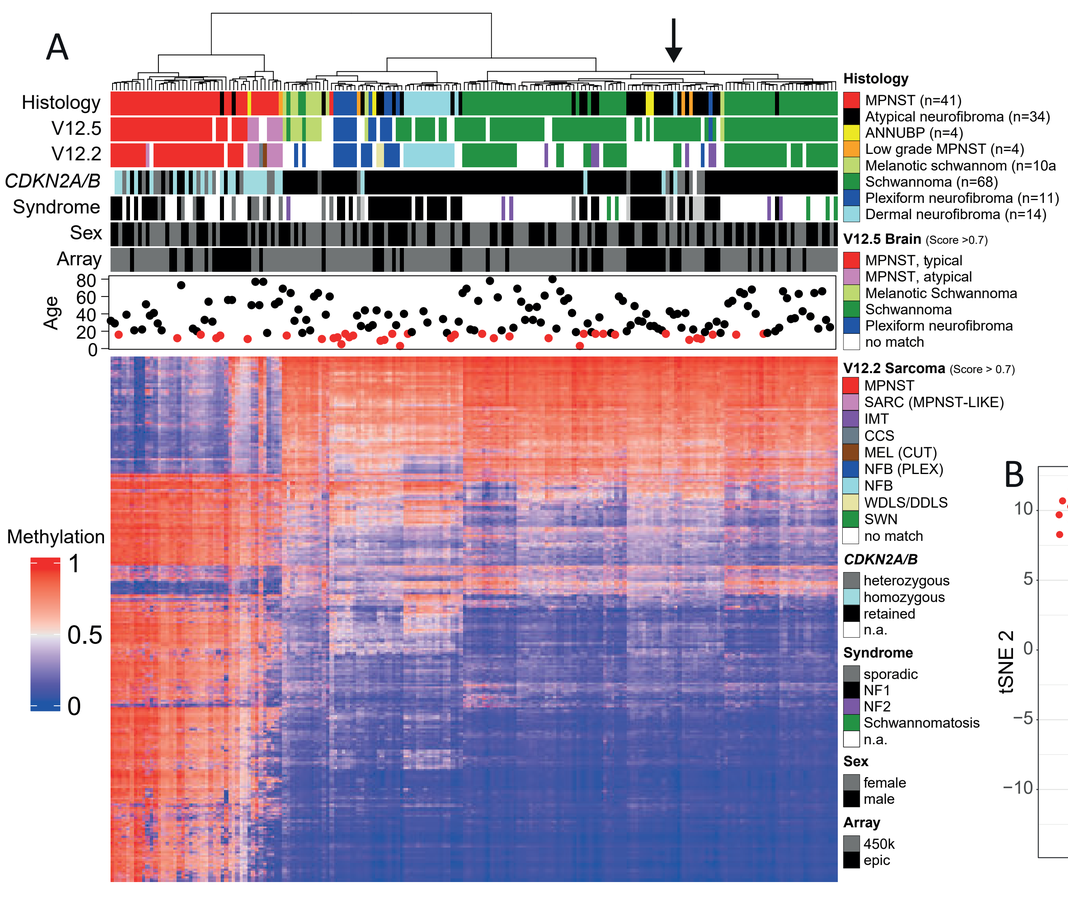
DNA methylation at CpG islands is crucial for gene expression and tissue-specific processes

Tumor DNA is isolated from FFPE material

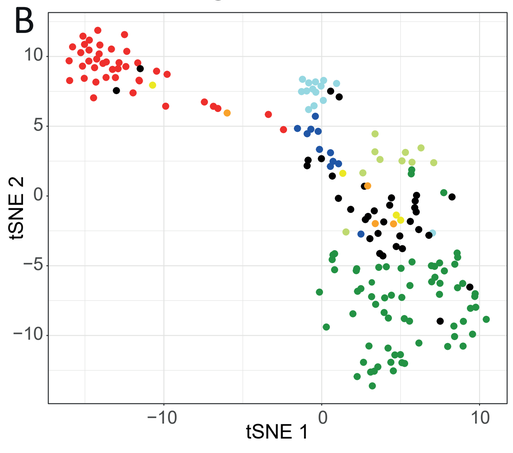
Array-based methylation measurement is conducted at single CpG-site level of 450k or 850k CpG-sites

3. Results

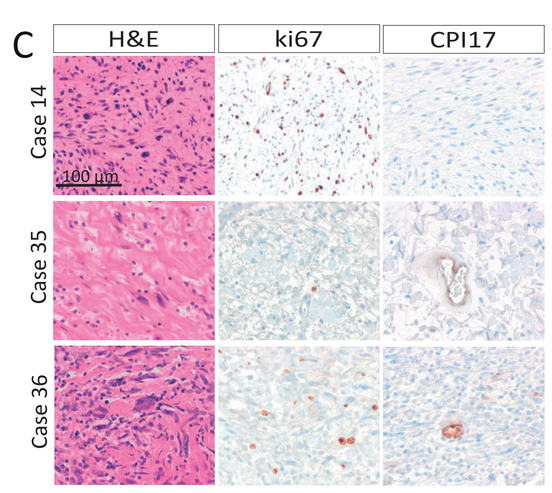
Methylation analysis reveals one main cluster of tumors diagnosed as atypical neurofibromas, ANNUBPs and low grade MPNST



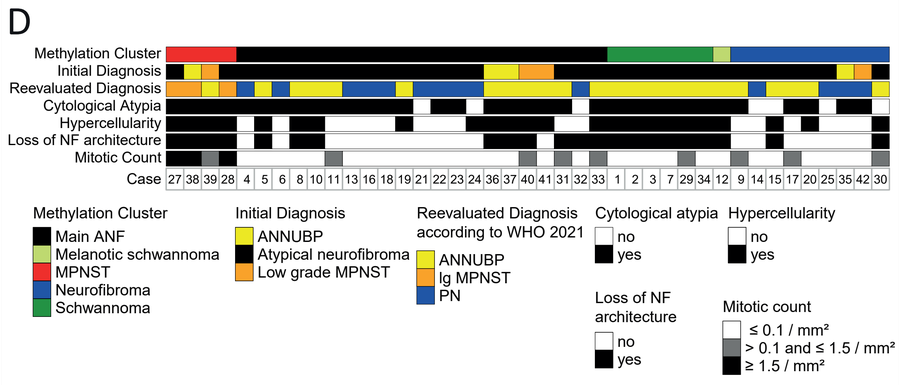
- 38/42 ANF cluster with benign entities of peripheral nerve sheath tumors
- 22 ANF form a distinct methylation cluster (arrow) and most receive a potentially misleading brain tumor classifier score for schwannoma. Most remain unclassifiable by the Sarcoma Classifier.
- 9 ANF cluster with bland NF
- 4 ANF cluster with MPNST, receive classifier scores for "atypical MPNST" and thus might have been underdiagnosed.



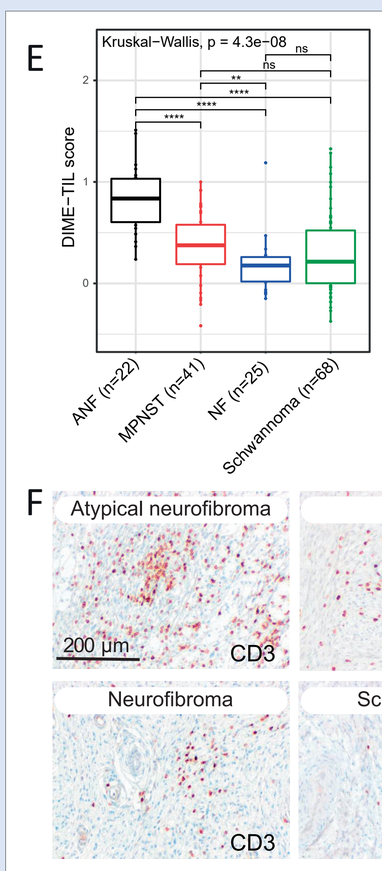
ANF histology is variable and their evaluation is rater-dependent



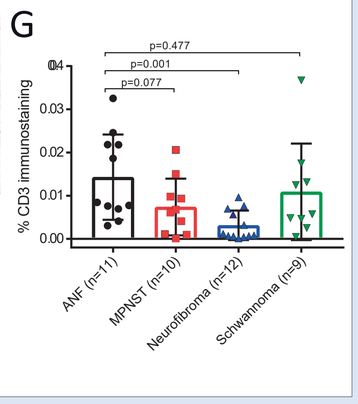
- Histologically diagnosed ANF have variable morphology regarding nuclear atypia, loss of neurofibroma architecture and proliferation indices.
- S100 is retained and CPI17
- Schwannoma marker CPI17 is negative in all tumor cells.
- Histological reclassification of ANF according to WHO 2021 critera shows discrepacies both to initial evaluation and methylation clustering



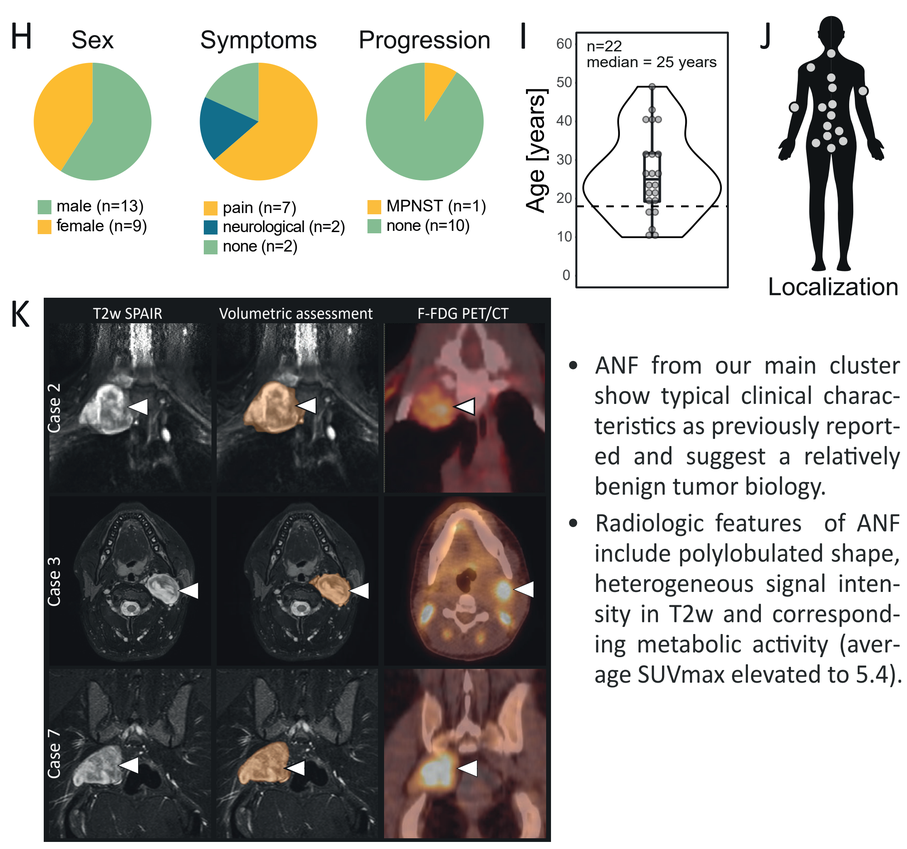
Methylome provides insight in immune signature



- DIMEImmune Scores calculated from global methylation data shows highest scores for tumor infiltrating lymphocytes (TIL), CD4- and CD8-positive lymphocytes in ANF (E)
- Digital image analysis of CD3 immunostaining signal supports highest level of immune infiltration in ANF



Clinical characteristics of ANF



- ANF from our main cluster show typical clinical characteristics as previously reported and suggest a relatively benign tumor biology.
- Radiologic features of ANF include polylobulated shape, heterogeneous signal intensity in T2w and corresponding metabolic activity (average SUVmax elevated to 5.4).

4. Conclusion

- Histological diagnosis of ANF is rater dependent and poses the risk of under and overdiagnosing tumors respectively.
- Array-based methylation data can help to stratify risk lesions based on cluster analysis.
- Most ANF have a common methylation profile that suggests a more benign tumor entity.
- ANF showed clinical characteristics comparable to previously published cases.
- Global methylation data can provide insight in immune infiltration and showed a significantly higher amount of tumor infiltrating lymphocytes in ANF compared to other PNST.

5. Declarations

I declare that I have no previous or ongoing business-related, personal, or commercial relations to industrial enterprises, or sponsors of medical institutions since 1st November 2021.