

Omics analysis to unveil Alveolar Rhabdomyosarcoma metastatic process

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BACKGROUND

Rhabdomyosarcoma (RMS) is the most prevalent soft tissue sarcoma (STS) in children and adolescents. It is divided in two major histological subtypes, being the Alveolar (ARMS) the one associated to a poorer prognosis¹. The cure rate for these tumors is up to 70% for children with localized disease, but this numbers decrease notably to a 20% 5-year survival rate for patients that show metastatic spreading. However, mechanisms leading to metastatic disease remain poorly understood. Thus, there is an urgent need to better understand this process, which will hopefully lead to novel targeted therapies and improved survival rates for these patients.

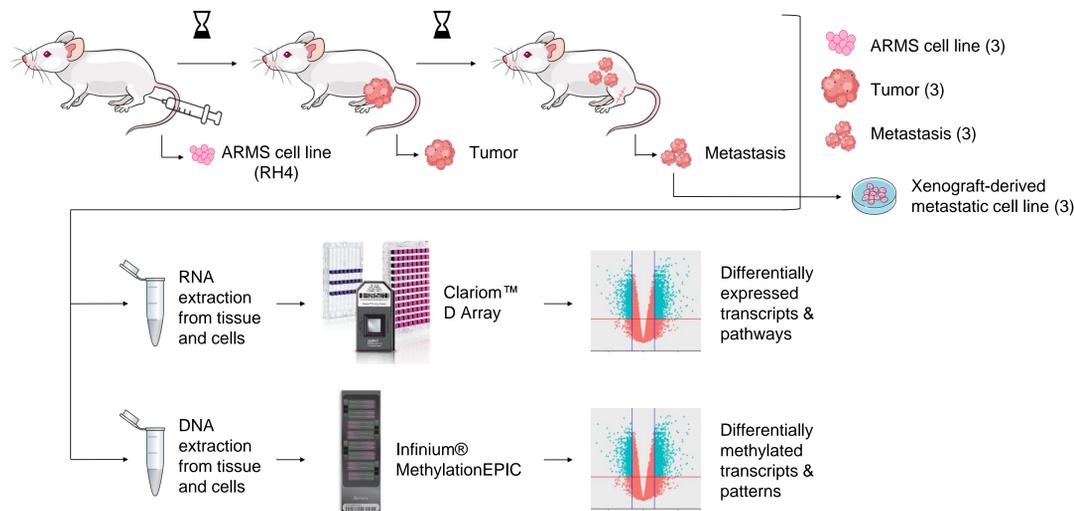


FIGURE 1. Pipeline of the study. ARMS cells (RH4) were injected in the gastrocnemius muscle of Athymic Nude-Foxn1tm mice². Primary tumors (800mm³) and peritoneal metastases (visible by IVIS[®]) were collected, and RNA/DNA extracted. Xenograft-derived metastatic cell lines were obtained directly as a primary culture from mice metastases. Characterization of paired tumors and metastases was performed using Clariom[™] D array (Applied Biosystems) and Infinium[®] MethylationEPIC (Illumina). After data normalization and comparative analyses (RMA and limma), a list of differentially expressed/methylated transcripts between sample groups was obtained.

1

SPONTANEOUS METASTATIC ORTHOTOPIC XENOGRAFT

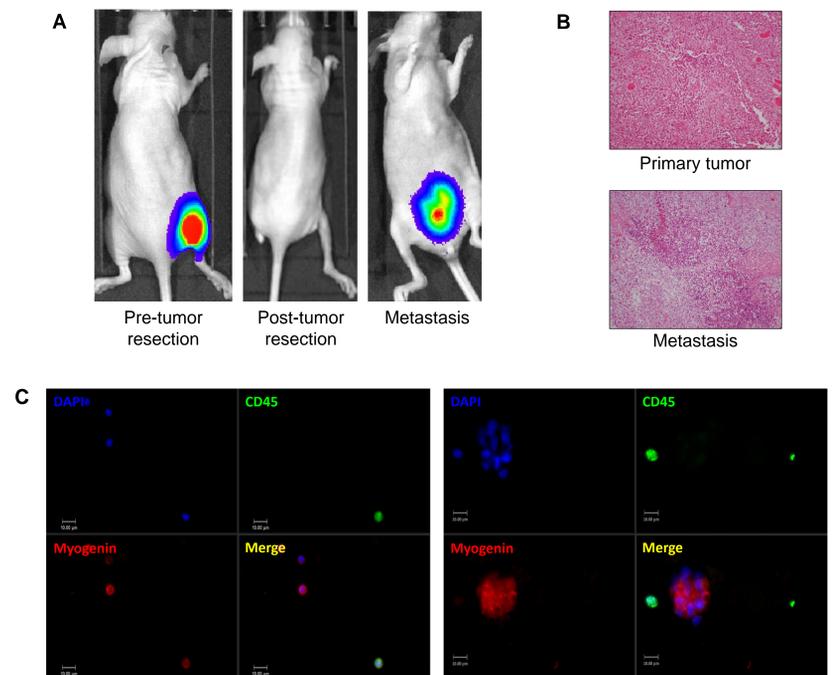
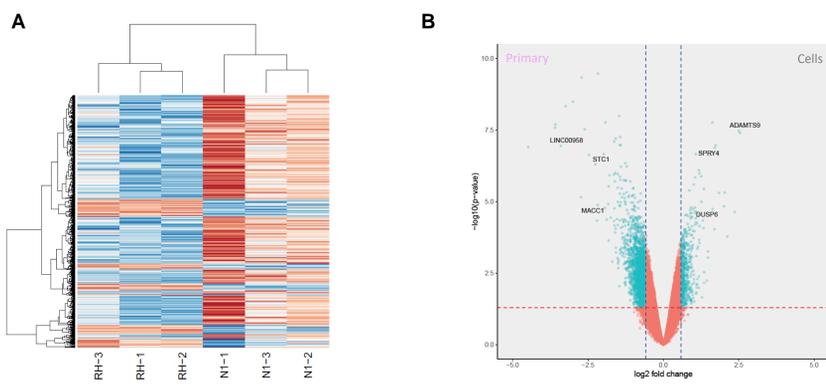


FIGURE 2. Spontaneous metastatic orthotopic ARMS mouse model developed by our group². A) Different stages of the ARMS tumor development in athymic (Nude-Foxn1tm) after injection of luciferase-labelled RH4 cell line. IVIS[®] detection of the tumor (~20 days after injection); absence of luminescence once the tumor is resected and detection of ARMS peritoneal metastatic mass (~50 days after injection) *in vivo*. B) Immunohistochemistry (H&E staining) of mice primary tumor and abdominal metastatic mass. C) Circulating tumor cells (CTCs) on mice blood detected by immunostaining. CTCs are detected as single cells (left) or clusters (left). DAPI used for nucleus staining, CD45 for lymphocytes and myogenin for ARMS cells.

2

TRANSCRIPTOMIC AND METHYLOMIC PROFILING OF ARMS SAMPLES IDENTIFIES POTENTIAL PRO-METASTATIC CANDIDATES

Transcriptomic profiling



Methylomic profiling

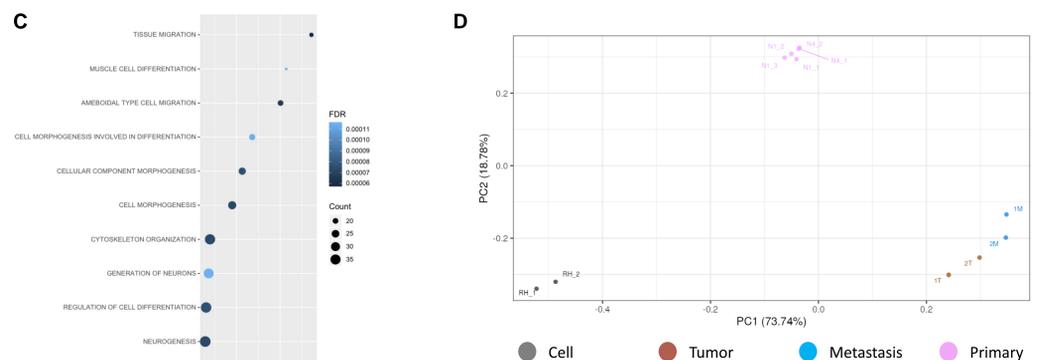


FIGURE 3. Transcriptomic and methylomic analyses on ARMS cell lines, tumors, metastasis and metastatic-derived primary cell lines reveal new candidates of disease progression. A) Transcriptomic profiling of ARMS mRNA samples (cells n=3, metastasis-derived primary cultures n=3) showed differential expression profile when comparing Cells vs. Primary. B) Volcano plot of transcriptomic (mRNA) results showing 2747 transcripts enriched in primaries (left, pink) compared to 673 transcripts enriched in cells (right, grey). Examples of DEGs are shown. C) Pathway analysis on primary-derived metastatic samples D) Principal component analysis (PCA) of methylome data was performed on RH4 cell line (n=3), tumors (n=2), metastases (n=2) and primary cell lines (n=5) to see how cells, tumors and metastases cluster. As seen, the origin of the sample (culture/tissue) accounts for most of the variation. Nevertheless, primary cells show clearly different profile than parental cells. Transcriptomic analysis performed on Clariom[™] D array. Methylomic analysis performed on Infinium[®] MethylationEPIC kit. Analysis of transcriptomics (data normalisation (RMA) and differential expression analyses (limma)) was performed with R. Threshold at adjusted p-value < 0.05, FC > 1.5. Analysis of methylomics was performed by external collaborators (IJC).

3

IN VITRO VALIDATION OF ARMS PRO-METASTATIC CANDIDATES

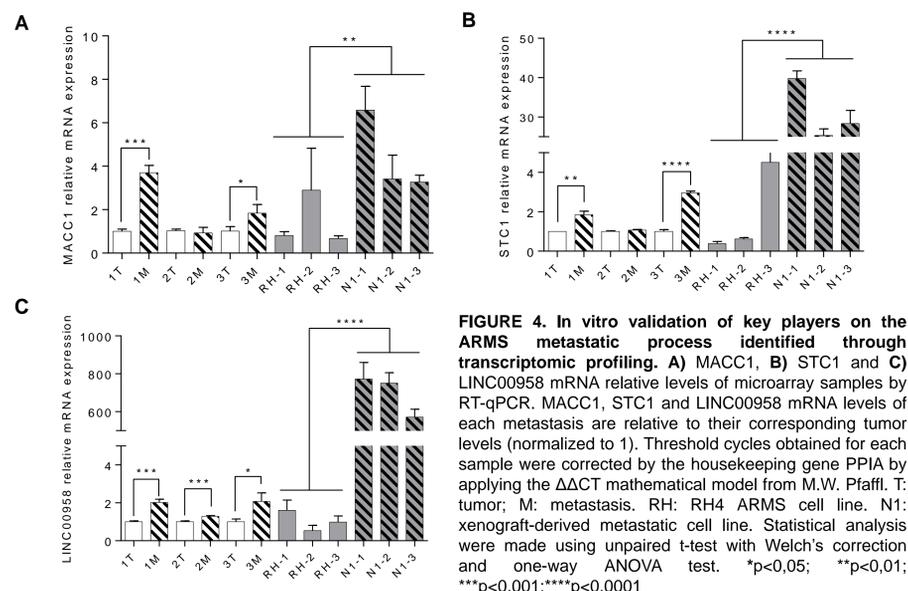


FIGURE 4. In vitro validation of key players on the ARMS metastatic process identified through transcriptomic profiling. A) MACC1, B) STC1 and C) LINC00958 mRNA relative levels of microarray samples by RT-qPCR. MACC1, STC1 and LINC00958 mRNA levels of each metastasis are relative to their corresponding tumor levels (normalized to 1). Threshold cycles obtained for each sample were corrected by the housekeeping gene PPIA by applying the $\Delta\Delta CT$ mathematical model from M.W. Pfaffl. T: tumor; M: metastasis. RH: RH4 ARMS cell line. N1: xenograft-derived metastatic cell line. Statistical analysis were made using unpaired t-test with Welch's correction and one-way ANOVA test. *p<0,05; **p<0,01; ***p<0,001; ****p<0,0001

CONCLUSIONS AND FUTURE WORK

- Analysis of the transcriptomic and methylomic profiles of ARMS samples allows the identification of candidate targets that could be key players in disease progression.
- ARMS cell lines and xenograft-derived metastatic cell lines show different expression and methylation profiles, indicating ARMS cells adaptative ability and modifications throughout tumor development.
- This observed differences are less clear in tissue samples, probably due to surrounding tissue contaminations.
- MACC1, STC1 and LINC00958 are enriched in metastasis samples and could be implicated in the metastatic potential of ARMS cells. These targets are related in literature to metastasis in other cancers, as well as lymph node metastasis, which happens in our model and in ARMS clinics³.
- Integration of both omics datasets will allow us further identification of putative ARMS metastasis regulators.

Studying the profile of paired tumors and metastases provides a unique tool to decipher the mechanisms of tumor progression

Understanding metastatic process → Better targeted therapies → Improved outcomes

References:

- [1] Skapek, S.X. et al. 2019, Nat Rev Dis Primers 5(1).
- [2] López-Aleman R, Tirado OM. 2021, Ewing Sarcoma Methods and Protocols. Vol 2226; 201-213.
- [3] Nishida, Y. et al. 2013, Int J Clin Oncol 19(3).