Histology-guided co-extraction of nucleic acids from archived tissues with limited tumor content: implementation in integrative epidemiologic studies

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Background

To improve the current knowledge of cutaneous melanoma and identify robust classifiers predictive of progression, we are conducting a multicenter study that will integrate data from 1,000 melanoma cases using limited archived primary melanoma for studies of gene and miRNA expression, methylation, somatic mutations, copy number variations, and selected protein expression. The small size and pigmentation of tissue present challenges, therefore systematic handling of tissues and co-extraction of nucleic acids from common tumor cell lysates are key to ensuring uniformity across specimens and proper integration of DNA and RNA-derived molecular findings across program projects.

Here we show the pilot study leading to this ongoing project and its current state.

Aims

To optimize the sectioning and handling of archived formalin-fixed, paraffin embedded (FFPE) tissues, and the histology-guided co-extraction, characterization, and quality control of nucleic acids for downstream measures of genetic and epigenetic events in FFPE primary tumors.

Approach

- Pilot set of 34 primary melanomas was obtained from two institutions of the InterMEL consortium.
- Pathology review and histology-guided nucleic acid co-extraction were conducted.
- DNA quality assessed via NanoDrop and Qubit. RNA assed via NanoDrop and Tapestation.
- Expression of 760 genes of interest in melanoma measured with NanoString platform; TCGA data used for validation.
- Two cases screened for somatic mutations in 410 genes using MSK IMPACT assay.

Results

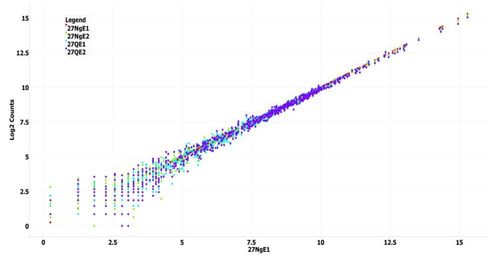

Figure 1. **Gene expression analysis:** The scatter plot shows comparative signals obtained from two independent extractions and two independent elutions of RNA (for fractions) from the same melanoma case. The scatter occurs for the low intensity points only.

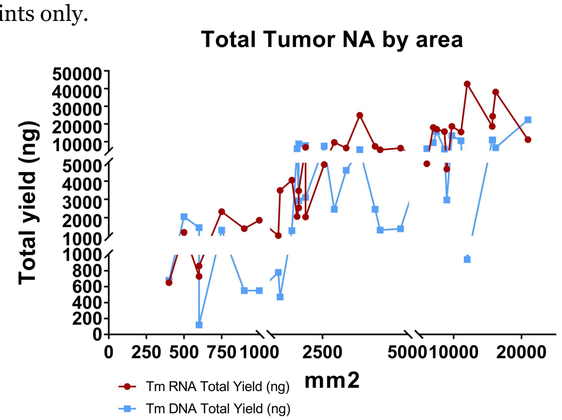

Figure 2. **Total Tumor NA by Area for Pilot cases:** Amount of nucleic acids obtained from each case of Melanoma Tumor relative to the tumor area.

Conclusion

- This work leads to the InterMEL study in progress.
- Pilot data indicate that nucleic acid co-extraction from limited archived tissues is feasible.
- In 94% of the pilot cases, DNA and RNA quantities were sufficient for parallel testing of somatic mutations, methylation, and gene and miRNA expression.

Acknowledgments

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Ongoing study: InterMEL

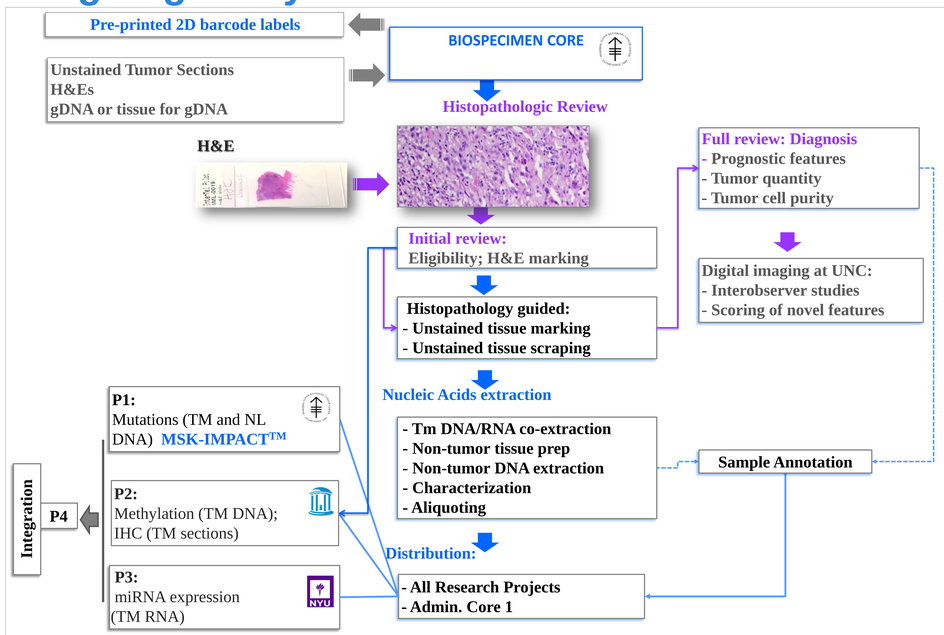

Figure 3. **InterMEL work-flow:** After validation of feasibility with the pilot study, the InterMEL study is ongoing following this approach. Nucleic acids from **1000** melanoma cases (obtained from 9 different centers) will be extracted, assessed for quality and studied using the tools described and optimized during the pilot study for this project.

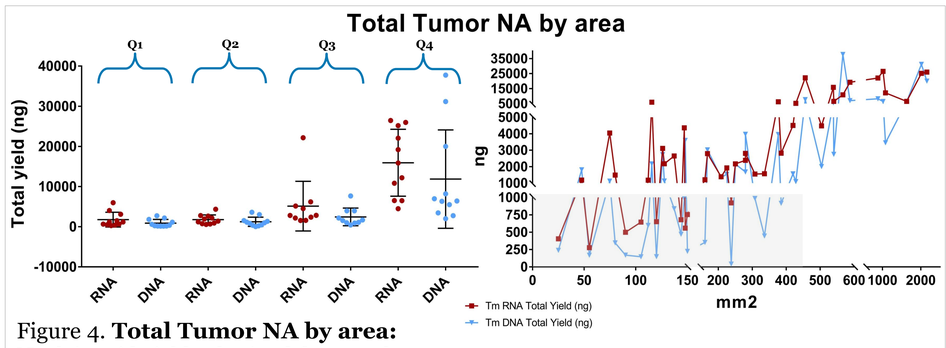

Figure 4. **Total Tumor NA by area:** Nucleic acid yields obtained from each case of Melanoma Tumor, relative to the total tumor area. On the left panel, area is separated into Quartiles. On the right panel, the nucleic acid yields are represented relative to the tumor area extracted.

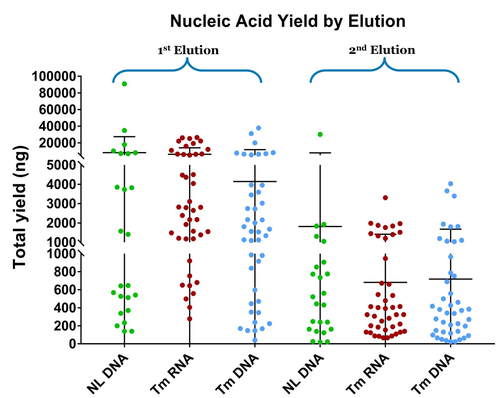

Figure 5. **Nucleic Acid Yield by Elution:** Comparison between the nucleic acids extracted from normal (NL) and tumor (Tm) cells from each case, as well as the yield obtained from the 1st and 2nd elution from the purification column.

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