Suitability of formalin-fixed paraffin-embedded tumor tissue for unbiased tumor associated antigen identification

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Master thesis in Bioinformatics and Computational Biology

For the effectiveness of targeted immunotherapy, which is currently one of the most promising treatments for brain tumors, it is necessary to know specific tumor-associated antigens (TAA). This project aimed to validate the suitability of formalin-fixed and paraffin-embedded (FFPE) material instead of fresh-frozen material for RNA sequencing and downstream TAA identification. A comparative analysis based on the RNA-seq of canine oligodendroglioma showed that formalin fixation of the samples had a significant effect on the RNA-seq library in terms of quality. Read distribution analysis showed that the FFPE samples contained fewer reads mapping to exonic regions and were enriched with reads mapped to introns and intergenic regions, while the fresh-frozen samples were mostly enriched in reads mapping to exons. Mismatch profile and SNVs calling analyses revealed some substitution artefacts present in the FFPE samples and absent in the corresponding fresh-frozen samples. However, according to the Principal Component Analysis, 36% of the variance between the samples could be explained by the types of conservation, while 43% of the variance could still be attributed to different expression profiles between oligodendroglioma and control conditions. A differential expression analysis of fresh-frozen oligodendroglioma versus fresh-frozen control samples identified 62 potential TAA strongly up-regulated in tumor tissue. The same analysis using the FFPE samples showed 80% of these genes (49/62) also to be differentially expressed. Comparative analysis showed good agreement between FFPE and fresh-frozen RNA-seq libraries in terms of the accuracy of gene expression measurements indicating that archived FFPE tissue can be used for identification of TAA candidates by RNA-seq providing a wealthy source of clinical samples for research. The following 10 genes encoding cell surface proteins have been identified by both approaches as potential TAA candidates based on their strong overexpression in oligodendrogliomas: PDGFRA, NOTCH1, DLL1, GPER1, TNR, IQGAP3, CD44, ERBB3, BCAS1 and ROR2. Future projects still need to investigate their suitability as TAA for targeted immunotherapy.

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