Transcriptomic modification induced by the first cycle of neoadjuvant chemotherapy impacts response to treatment in triple negative breast cancer (TNBC)

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Background

Despite adequate neoadjuvant chemotherapy, TNBC remains poor prognosis. Non-responding patient have 25-40% risk of relapse at 5 years. pCR is therefore currently considered as a major goal in TNBC and new tools of early prediction of residual disease should be identified.
TransTep is a phase 2 monocentric clinical trial which aims to identify transcriptomic profile of triple-negative cancer cells associated with early tumor chemoresistance, as identified by FDG PET after the first course of neoadjuvant chemotherapy.

Materials and methods

Population

Twenty patients were included between January 2015 and October 2017, with stage II or III of the UICC classification (except stage T4d). All patients received neoadjuvant chemotherapy with anthracyclines and taxanes sequentially, none of them had a dose-dense chemotherapy. Six patients obtained metabolic response (Delta SUV < -50%) after one cycle of anthracycline.

RNA extraction

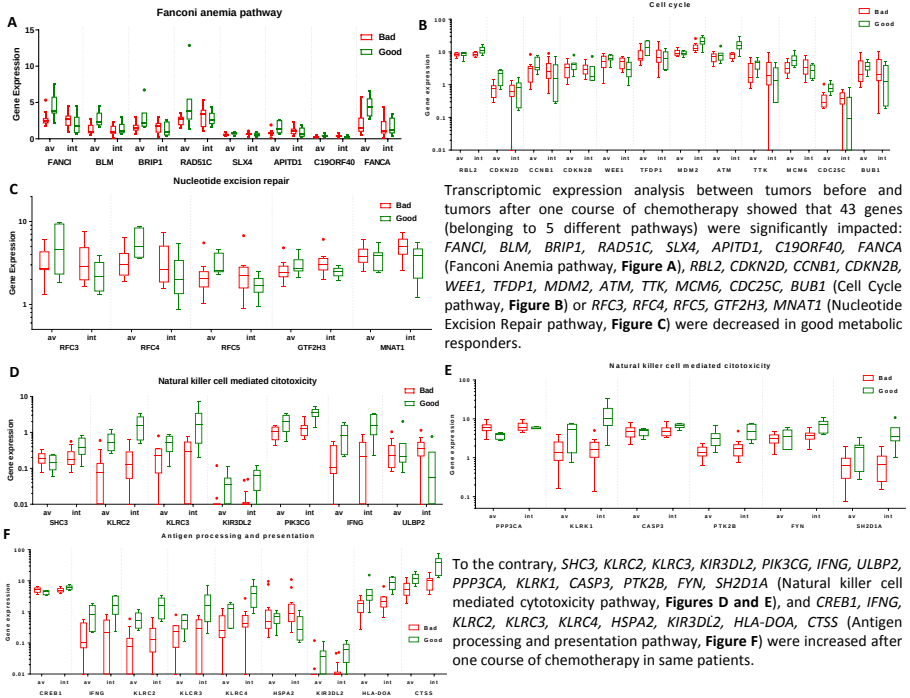
RNA was extracted using the Maxwell-16 LEV RNA formalin-fixed paraffin-embedded Purification kit (Promega) according to the manufacturer's protocol. RNA quality and quantity were assessed by spectrophotometry with absorbance at 230, 260 and 280 nm.

RNA sequencing

RNA depleted of ribosomal RNA was used for the library preparation with a TruSeq RNA library Prep kit according to the manufacturer's instructions (Illumina). RNA
sequencing was performed on a NextSeq500 device (Illumina). The libraries were sequenced with paired-end 76–base pair 'reads'. Analysis was performed with the
splice junction mapper TopHat for Illumina on the human Hg19 genome.

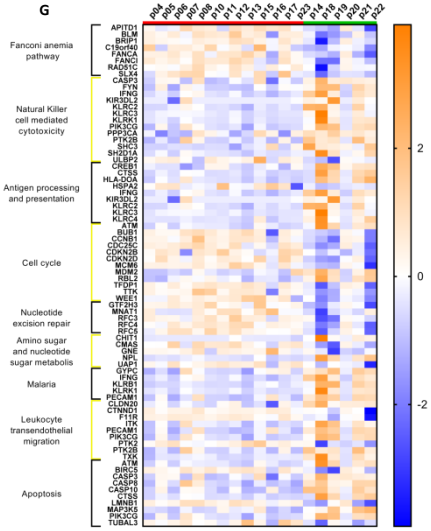
RNA-Seq data were aligned to the transcriptome and reference genome with TopHat 2.1.1. The reference genome was downloaded on the UCSC (University of California, Santa Cruz) Genome Browser (hg19). Fragments per kilobase of transcript per million (FPKM) were calculated using Cufflinks 2.2.1. Differential genes were selected using the Cuffdiff program from Cufflinks through the cummeRbund R package. Only genes with the false discovery rate-adjusted p-value under 0.05 were kept.

Results



Transcriptomic expression analysis between tumors before and tumors after one course of chemotherapy showed that 43 genes (belonging to 5 different pathways) were significantly impacted: *FANCI, BLM, BRIP1, RAD51C, SLX4, APITD1, C19ORF40, FANCA* (Fanconi Anemia pathway, **Figure A**), *RBL2, CDKN2D, CCNB1, CDKN2B, WEE1, TFDP1, MDM2, ATM, TTK, MCM6, CDC25C, BUB1* (Cell Cycle pathway, **Figure B**) or *RFC3, RFC4, RFC5, GTF2H3, MNAT1* (Nucleotide Excision Repair pathway, **Figure C**) were decreased in good metabolic responders.

To the contrary, *SHC3, KLRC2, KLRC3, KIR3DL2, PIK3CG, IFNG, ULBP2, PPP3CA, KLRK1, CASP3, PTK2B, FYN, SH2D1A* (Natural killer cell mediated cytotoxicity pathway, **Figures D and E**), and *CREB1, IFNG, KLRC2, KLRC3, KLRC4, HSPA2, KIR3DL2, HLA-DOA, CTSS* (Antigen processing and presentation pathway, **Figure F**) were increased after one course of chemotherapy in same patients.



In addition to the 43 genes belonging to the 5 pathways previously discussed, we also observed that 23 other genes belonging to sugar metabolism, leukocyte transendothelial migration and apoptosis are impacted by the 1st cycle of chemotherapy and that this expression variation showed a link with the metabolic response after one cycle with anthracyclines (**Figure G**).

Conclusion

These first results need to be correlated with the pCR data. In case of residual disease detection, a new trial that will propose therapeutic adaptation to the metabolic and transcriptomic data will be proposed to increase pCR rate and prognosis.

Contact

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