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Title page

Title: Whole Blood Gene Expression Profiling in Patients undergoing Colon Cancer Surgery identifies Differential Expression of Genes involved in Immune Surveillance, Inflammation and Carcinogenesis

Running head: Gene Expression after Colon Cancer Surgery

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Abstract

Introduction

Cancer surgery may represent a potential risk of enhanced growth and metastatic ability of residual cancer cells due to post-operative immune dysfunction. This study identifies changes in transcription of genes involved in immune surveillance, immune suppression and carcinogenesis in the post-operative period of laparoscopic colon-cancer surgery within an ERAS regime.

Methods

Patients undergoing elective, curatively intended laparoscopic surgery for colon cancer stage I-III UICC were included in the study. Patients followed standard of care in an ERAS setting. Whole blood gene expression profiling (WBGP) was performed on the day prior to surgery and 1, 2, 3 and 10-14 days after surgery. Samples were collected in Paxgene tubes and Labeled cDNA was fragmented and hybridized to Affymetrix GeneChip™ 2.0.

Results were corrected for multiple hypothesis testing using the false discovery rate. Pathway analysis was performed through the Molecular Signature Database. Paired fold changes of gene expression were calculated for post-operative compared to pre-operative samples. A mixed effect model was used to test differential gene expression by repeated-measures ANOVA.

Results

WBGP of 33,804 genes at five timepoints in six patients showed 302 significantly differentially expressed genes between samples from the day prior to surgery and the day after surgery. Pathway gene enrichment analysis showed a downregulation of immunologically relevant pathways. There was a significant downregulation of genes involved in T-cell receptor signalling, antigen
presentation and NK-cell activity after surgery. Furthermore, there was an upregulation of cytokines related to metastatic ability, growth and angiogenesis.

Conclusion

Whole blood gene expression profiling revealed dysregulation of genes involved in immune surveillance, inflammation and carcinogenesis, after laparoscopic colon cancer surgery.

**Manuscript text**

**Introduction**

Colorectal cancer (CRC) is the third most common malignancy in the world, accounting for over 600,000 deaths annually\(^1\). Surgery is essential for cancer treatment\(^2\), and surgical removal of the tumor is mainstay of treatment in a curative treatment strategy\(^3\).

Growing evidence supports that surgery\(^4\) and the post-operative period\(^5\) contributes to increased risk of cancer relapse, caused by surgical stress response\(^6\). Surgery causes shredding of tumor cells\(^7\), and micrometastasis in lymph vessels or other tissues might be present in spite of a negative surgical margin\(^8\). Cancer surgery may represent a potential risk of enhanced growth and metastatic ability of these residual cancer cells\(^9\). The surgical stress response with activation of the sympathetic nervous system\(^10,11\) causes impaired cellular immune function\(^12\) and NK-cell activity\(^13\). Furthermore, both the surgical trauma\(^14\) and anesthetic agents\(^15\) facilitate growth and metastatic ability. By these mechanisms, factors related to the surgical stress response may cause a risk of cancer-relapse\(^16\).

Post-operative immune function after colon cancer surgery (LAC), has been addressed by several studies\(^17\), and evidence points in the direction that LAC causes impaired immune function, though maybe not as severe as in open surgery\(^14\).
As evidence is accumulating, that laparoscopic assisted colectomy (LAC) results in reduced morbidity\textsuperscript{18}, shorter length of hospital stay and better Quality of Life\textsuperscript{19}, compared to open surgery, LAC should be considered for colon cancer surgery, and there is a need for thorough investigation of exact changes within immunological surveillance and efficiency after LAC\textsuperscript{17}.

Enhanced recovery after surgery (ERAS) regimes have been implemented in recovery after surgery for colo-rectal cancer\textsuperscript{20} and resulted in reduced post-operative complications and shorter length of hospitalization\textsuperscript{21,22}. Evidence of effect on post-operative cellular immunity and systemic inflammatory response within an ERAS regime is limited\textsuperscript{23,24,25}.

Cellular immunity is becoming increasingly recognized as a factor related to survival of cancer patients\textsuperscript{26} as seen in the association between lymphocytic invasion of resected tumors and survival\textsuperscript{27,28}. There is a growing interest and success with immunotherapy for cancer in general\textsuperscript{29}, and in small clinical trials with pre-operative immune-stimulation\textsuperscript{30,31}. With increased knowledge of the complex repertoire of immunological cells and cytokines affected by LAC, per-operative therapeutic opportunities could be exploited further for these potentially curable patients\textsuperscript{32}.

Whole Blood Gene Expression Profiling (WBGP) offers a unique possibility to obtain global knowledge of the complex immune response following colon cancer surgery and gain insight in transcription of multiple genes in all peripheral immune cells\textsuperscript{33,34}. It is possible to collect a large amount of data regarding changes in gene transcription of cytokines, surface-molecules, intra and extracellular transcription factors in all immune cells, with few sampling methods and pre-analytical processing steps. The aim of this study was to identify changes in transcription of genes involved in immune surveillance, immune suppression and carcinogenesis in the post-operative period in a cohort of patients undergoing laparoscopic colon-cancer surgery within an ERAS regime.
Methods

Participants

From January to July 2016, patients undergoing elective, curatively intended laparoscopic surgery for colon cancer at Zealand University Hospital were enrolled consecutively, based on the following criteria: A diagnosis of colon cancer stage I-III Union for International Cancer Control (UICC), laparoscopic surgery and anesthetics with total intravenous anesthetics (TIVA). Patients undergoing neoadjuvant radio- or chemotherapy, or patients with known immune defects, a history of previous cancer, benign tumors or patients who experienced any post-operative complications, were excluded from the study.

Settings

All eligible patients received information regarding purpose and methods of the study and were included after giving oral and written consent.

During the perioperative period, patients followed standard of care for colon cancer in an ERAS setting, including early mobilization, early enteral high-protein intake, breathing exercise, physiotherapy and sufficient pain management. No restrictions were imposed on pain management or surgical approach. For induction of anesthesia, propofol 2-3 mg/kg and a short acting opioid (remifentanil or sufentanil) were administered. Hereafter they received a continuous infusion of propofol 5 mg/kg/h supplemented with a controlled infusion of remifentanil 0.5 µg/kg/min. Patients received ondansetron 4 mg and sufentanil 0.4-0.6 µg/kg 20-30 minutes before extubation supplemented with 1 g paracetamol. Local anesthesia (ropivacaine) was administered in the wounds.

Data collection and processing
Demographic data was collected through the electronic patient chart including age, gender, smoking status, body mass index (BMI), American Society of Anesthesiologist (ASA) scores and Charlson Comorbidity Index (CCI).

The UICC stage was based on pre-operative CT scans and histology results.

Blood samples were taken on the day prior to surgery and on day 1, 2, 3, and finally, between 10 and 14 days after surgery.

Samples were collected in Paxgene tubes (Preanalytix, Hombrechtikon, Switzerland) and stored at room temperature for 24 hours, then at −20°C for a minimum one day, and finally transferred to a −80°C freezer. Total RNA was extracted from each sample using the Paxgene Blood RNA kit (Qiagen, Franklin Lakes, NJ, USA). The quantity and quality of RNA were tested with a NanoDrop spectrophotometer ND-8000 (NanoDrop Technologies) and an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA), respectively.

The GeneChip™ WT PLUS Reagent Kit was applied to convert 500 ng of purified total RNA to biotin-labeled cDNA. Labeled cDNA was fragmented and hybridized to Affymetrix GeneChip™ Human Transcriptome Array 2.0.

Background correction, normalization, and gene expression index calculation of probe intensities were done in Affymetrix Expression Console software using the robust multi-array average (rma) method. The regularized t-test limma for paired data was applied to calculate the significance of the difference in gene expression between pre and post-operative samples. All p-values were corrected for multiple hypothesis testing using the false discovery rate (FDR). An FDR < 0.05 was considered significant. Genes chosen for validation analysis were based on review of current literature in order to cover clinically relevant immunological pathways described previously, in regards to the surgical stress response.
Pathway analysis was performed through the Molecular Signature Database (http://software.broadinstitute.org/gsea/msigdb) using Reactome Pathways\textsuperscript{39}. This is an overrepresentation analysis analyzing significance of overlap with Reactome gene sets using hypergeometric statistics followed by correction for multiple testing using the FDR method.

Paired fold changes of gene expression were calculated for post-operative samples compared to pre-operative samples. The gene expression fold change matrix was visualized by heat maps using the heatmap.2 function embedded in the \textit{gplots} R-package.

A mixed effect model was used to test differential gene expression by repeated-measures ANOVA, as implemented in the \textit{lme} function embedded in the \textit{nlme} R-package\textsuperscript{40}.

The Central Committee for Health Research and Ethics (file no: 2008-58-0020) and the Danish Data Protection agency (protocol: SJ567) approved the study.

**Results**

Six patients were included (see Table 1 for demography). None of the patients had post-operative complications, blood transfusions or were treated with known immune-suppressive drugs in the period. All patients were admitted to hospital on the day prior to surgery and discharged 2-3 days after surgery.

Analysis of 33,804 genes by WBGP showed 302 significantly differentially expressed genes after correction for multiple comparisons (FDR<0.05) in samples from the post-operative day 1 (POD1), compared to pre-operative blood samples. Table 2 showed fold changes (FC), unadjusted p-values and FDR of the top 20 most significantly down or up-regulated genes. Many of these genes were related to immune function. To gain more insight in which pathways were represented amongst the top differentially expressed genes, a pathway gene enrichment analysis of the 100 most significantly
differentially up and downregulated genes between the pre-operative and POD1 was performed (Table 3), showing an overrepresentation of immunologically relevant genes in the significantly downregulated pathways.

There were no significantly differentially expressed genes on POD-2, 3 or 10 compared individually to pre-operative expression, when corrected for multiple comparisons for all genes (FDR ≥ 0.05). To clarify whether gene expression values followed a relevant time-course pattern, ANOVA was performed on all genes. The associated ANOVA significance levels for candidate-genes are listed in Table 4.

To visualize the time-course changes in gene expression, and analyze for hierarchical clustering of candidate genes in the post-operative period, a heatmap, based on FC-values between the day prior to surgery and POD 1, 2, 3 and 10, was created (Figure 1). This showed a two-sided clustering in genes up- or downregulated after surgery. Genes relevant for antigen presentation (MHC-I and II), general TCR (T-cell receptor) signaling (CD3, CD4 and CD8) and Granzyme-B (GZMB) encoding for NK and CD8⁺ T-cells cytotoxic molecule were all in the downregulated cluster which also included interferon gamma encoding gene (IFNG) and TP53 encoding p53 tumor suppressor protein. In the upregulated cluster, genes were encoding for inflammatory cytokines (CRP, IL1B, IL6, TNF), immunosuppressive cytokine IL10, growth and angiogenic-factor encoding genes (VEGFA, C, EGF, EGFR), and matrix metalloproteinase encoding genes (MMP2 and 9).

Candidate genes were chosen prior to analysis, and the individual ANOVA unadjusted p-values and FDR values for these genes were presented in Table 4, accompanied with a short description of gene function in relation to post-operative stress and cancer immune surveillance.

Discussion
In six patients undergoing laparoscopic surgery for colon cancer within an ERAS regime, we found significant deregulation of genes involved in cellular immune-functions, and tumor suppression.

Pathway analysis

In the gene enrichment pathway analysis of the 100 most up and downregulated genes on the first POD, there was a significant downregulation of pathways involved in antigen presentation, T-cell activation, differentiation and development of T-cells and lymphocyte activation. Downregulation of genes involved in T-cell recognition of pathogens is especially unwanted during this period, as cancer cell immune evasion is thus potentiated\(^\text{41}\). Genes involved in interferon gamma (IFN\(\gamma\)) signaling was also significantly downregulated in the first POD. IFN\(\gamma\) is crucial for an immune response and related to both NK and cytotoxic T-cell activity. It has direct anti-tumor effects on cancer cells and indirect anti-tumor effects through immune activation\(^\text{42}\), and its role in protection against tumor development is well described\(^\text{43,44}\). Downregulation of genes involved in this pathway during the immediate post-operative period with circulating tumor cells and residual micro-metastatic foci is unwanted, and previous studies with IFN-treatment prior to CRC-surgery has shown beneficial immunological outcomes\(^\text{30}\).

Based on previous research on post-operative immune suppression, candidate genes were chosen for heatmap clustering, which showed an interesting two-sided clustering with genes needed for immune surveillance and tumor suppression in one cluster which were downregulated after surgery, and a second cluster of genes which were upregulated including immunosuppressive and inflammatory cytokines, and angiogenic factors (Figure 1).

TCR-genes chosen as candidate genes were all downregulated on the first POD. Two of four genes encoding for CD3 TCR were significantly downregulated with FDR<0.05 (Table 4). T-cell density of CD3, 4 and 8\(^{+}\) T-cells in colorectal tumors is correlated to both disease-free survival\(^\text{27,45}\) and

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overall survival\textsuperscript{46}. A correlation between numbers of CD3 and 8\textsuperscript{+} T-cells in pre-operative blood-samples and infiltrating lymphocytes of removed tumors from patients undergoing surgery for CRC has been found in a recent study\textsuperscript{47}. CD3, 4 and 8 surface molecules of T-cells are associated with lck tyrosine kinase which activates ZAP-70 that is instrumental in initiating the intracellular pathways needed for T-cell activation, which was also found significantly downregulated in the reactome-pathway analysis.

MHC-I and II molecules are essential for the immune system to recognize self and non-self and essential for presentation of engulfed cancer-cells to be killed by the immune system\textsuperscript{48}. Previous studies have found post-operative downregulation of HLA-DRA after CRC surgery, with higher HLA-DRA after laparoscopic than open surgery\textsuperscript{49}. WBGP showed downregulation of all candidates genes related to both MHC I and II, indicating a possibility of cancer cell immune evasion in this critical period\textsuperscript{50}.

\textit{NK-cell dysfunction}

It is well recognized that surgery causes NK-cell dysfunction\textsuperscript{13,51}. NK cells are able to recognize and kill virus-infected cells or tumor cells, without preceding sensitization\textsuperscript{52}. GZMB encodes for granzyme B, which is crucial for the rapid induction of target cell apoptosis through NK-cell activity. WBGP revealed a significant decrease in GZMB expression in the first POD (FDR=0.05, ANOVA p-value=0.04), indicating NK-cell dysfunction after laparoscopic colon cancer surgery. Few clinical studies have examined NK-cell dysfunction after laparoscopic colon-cancer surgery, but one study examining immunological effects of laparoscopic versus open colorectal surgery found a smaller decline in numbers of NK-cells after laparoscopic than open surgery\textsuperscript{53}.

\textit{Insufficient antitumor immunity in the acute phase response}
The acute phase response initiated directly after or during surgery includes elevated pro-inflammatory cytokines IL1β, TNFα and IL-6\textsuperscript{54}. These are counteracted by immunosuppressive cytokines such as IL1β receptor antagonist and IL10\textsuperscript{32}. Randomized clinical trials examining post-operative immune status after open vs laparoscopic surgery have indicated lower IL-6 and CRP levels after laparoscopic surgery\textsuperscript{14}, but in regards to immunosuppressive cytokines IL1β receptor antagonist and IL10, results are conflicting\textsuperscript{17,55}. WBGP showed a post-operative upregulation of all genes encoding for the pro-inflammatory cytokines in heatmap clustering. FDR values showed a significant for upregulation of IL1β, but insignificant for CRP and IL6. Genes encoding IL10 and IL1β receptor antagonist showed a significant upregulation of both genes (FDR<0.04). The findings suggest that the acute phase response in the post-operative period is not effectively initiating antitumor immunity.

Enhanced tumor growth and invasion

In-vitro and animal studies have demonstrated that post-operative plasma enhances tumor-cell growth\textsuperscript{56}. This may partly be explained by elevated growth factors such as epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) which facilitates post-operative wound healing and angiogenesis\textsuperscript{57}. Previous studies have found a lower increase of these growth factors after laparoscopic than open surgery\textsuperscript{58}. Matrix metalloproteinases (MMP) induce breakdown of extracellular matrix and play an important role in tumor cell invasion and metastasis, by facilitating entry of tumor cells into the bloodstream, angiogenesis, especially MMP9\textsuperscript{59}. WPGP showed significant upregulation of the gene encoding for MMP9, implying the possibility for cancer cell-extravasation, invasion and metastasis.

Impaired tumor suppression
When activated, p53 can arrest the cell cycle, induce apoptosis, or promote senescence, exerting a protective effect on individual cells and induce suppression of migration, invasion and metastasis of colorectal cancer cells.\textsuperscript{60} WBGP showed a significant downregulation of the TP53 gene expression, indicating that the tumor suppressive effect of p53 is absent during the post-operative period.

There are several limitations to the study. First, there are only six patients included, making a generalization difficult. Furthermore, we have no knowledge of gene expression in subgroups of immunological cells, as transcription analysis was performed on whole-blood. Also, in this study, choosing single candidate genes as a focus might not be entirely right, as seen in an insignificant downregulation of the IFN\(\gamma\) gene, but pathway analysis found IFN\(\gamma\) signaling as one of the most significantly downregulated pathways. Of note, however, despite the limited number of patients included, we were able to unravel significant deregulation of genes that are considered to be of importance in regard to immunosuppression, inflammation, cancer invasiveness and metastasis.

Several studies have found that perioperative events, can influence cancer recurrence risk after surgery.\textsuperscript{61} Most randomized studies investigating the surgical stress response after open versus LAC, find a better preserved post-operative immune function after LAC\textsuperscript{14,24,49,55}. One randomized study found a lower risk of tumor recurrence and better overall survival in LAC versus open surgery\textsuperscript{62}, but whether this is related to better preserved post-operative immune function is not known. Studies investigating pre or post-operative immune function in relation to long term oncological outcome, indicate a connection between poor outcome and high pre-operative inflammation\textsuperscript{63}, magnitude of surgical stress response\textsuperscript{6}, and high post-operative inflammation\textsuperscript{64}. This study only includes patients undergoing laparoscopic surgery, and though this procedure is expectedly followed by less post-operative stress, a future study with comparison of changes in WBGP in open surgery would be interesting.
Whether interventions in the peri-operative period can enhance immunological status or decrease inflammation after colorectal cancer surgery and affect long term outcome, has been exploited in several retrospective\textsuperscript{65} and pre-clinical studies\textsuperscript{66,67}, and should also be exploited in future large randomized studies\textsuperscript{32} investigating effects on survival and residual cancer. Patients who underwent neoadjuvant chemotherapy were not included in this study, but it is possible, that neoadjuvant chemotherapy in patients undergoing non-metastasized surgical resection could be beneficial\textsuperscript{68}, and there ongoing clinical trials (NCT00647530).

Small non-randomized clinical trials and preclinical research have shown promising results, in regards to modulating the stress-response and immune system effects with different intervention in prior to CRC surgery. Preclinical\textsuperscript{69,70} studies have investigated the effect of pre-operative β-blockers and COX2 inhibitors. Studies are indicating these interventions designed to dampen the sympathetic nervous system and hence, the surgical stress response could be beneficial in reducing post-operative cancer risk\textsuperscript{71}. There are ongoing clinical trials investigating this further (NCT00888797).

Other pre-operative immunological interventions that have shown promising results in pre-clinical studies and small clinical trials are histamine agonists\textsuperscript{72,73} and interferon-α\textsuperscript{30,74}, but long term outcomes of large clinical trials are still lacking. There is a need for clinical studies with further investigation of these promising pre-clinical intervention studies, to examine whether pre-operative interventions can reduce the risk of residual cancer. WBGP might be a useful tool to obtain an integrated signature of possible beneficial effects of therapeutic interventions in regards to their impact upon enhancement of immune function and dampening of inflammation.
References


44. Parker BS, Rautela J, Hertzog PJ. Antitumour actions of interferons: implications for cancer


51. Ogawa K, Hirai M, Katsube T, Murayama M, Hamaguchi K, Shimakawa T, Naritake Y,


58. Belizon A, Balik E, Feingold DL, Bessler M, Arnell TD, Forde KA, Horst PK, Jain S, Cekic V, Kirman I, Whelan RL. Major abdominal surgery increases plasma levels of vascular


**Figure legend**

Table 1: Patient demographics.

Table 2: Top 20 of the most down and up-regulated genes in samples from post-operative day 1 compared to pre-operative.

Table 3: Pathway analysis of the 100 most up and downregulated genes in samples from post-operative day 1 compared to pre-operative.

Table 4: Fold change values for candidate genes in samples from post-operative day 1 compared to pre-operative.

Figure 1: Heatmap of foldchanges in candidate gene expression in samples from post-operative day 1, 2, 3 and 10 compared to pre-operative.
Table 1: Patient demographics

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ASA: American Society of Anesthesiologists-score; BMI: Body Mass Index; CCI: Charlson Comorbidity Index; UICC: Union for International Cancer Control
Table 2: Top 20 of the most down and up-regulated genes in samples from post-operative day 1 compared to pre-operative

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<td>2.7</td>
<td>2.7E-05</td>
<td>0.03</td>
</tr>
<tr>
<td>NKG7</td>
<td>-1.5</td>
<td>1.3E-05</td>
<td>0.03</td>
<td>PFKFB3</td>
<td>2.3</td>
<td>2.8E-05</td>
<td>0.03</td>
</tr>
<tr>
<td>OAS2</td>
<td>-1.7</td>
<td>1.5E-05</td>
<td>0.03</td>
<td>TGFA</td>
<td>1.6</td>
<td>2.5E-05</td>
<td>0.03</td>
</tr>
<tr>
<td>HLA-DRB4</td>
<td>-1.7</td>
<td>1.6E-05</td>
<td>0.03</td>
<td>AGPAT9</td>
<td>1.5</td>
<td>3.3E-05</td>
<td>0.03</td>
</tr>
<tr>
<td>IGHV3-64</td>
<td>-1.5</td>
<td>2.5E-05</td>
<td>0.03</td>
<td>KLLN</td>
<td>1.4</td>
<td>2.9E-05</td>
<td>0.03</td>
</tr>
<tr>
<td>CD74</td>
<td>-1.6</td>
<td>3.5E-05</td>
<td>0.04</td>
<td>LOC731424</td>
<td>1.7</td>
<td>2.6E-05</td>
<td>0.03</td>
</tr>
<tr>
<td>FAIM3</td>
<td>-1.7</td>
<td>3.9E-05</td>
<td>0.04</td>
<td>MMP9</td>
<td>2.7</td>
<td>2.7E-05</td>
<td>0.03</td>
</tr>
<tr>
<td>SPN</td>
<td>-1.5</td>
<td>3.8E-05</td>
<td>0.04</td>
<td>PFKFB3</td>
<td>2.3</td>
<td>2.8E-05</td>
<td>0.03</td>
</tr>
<tr>
<td>TRAI1</td>
<td>-1.8</td>
<td>4.1E-05</td>
<td>0.04</td>
<td>TGFA</td>
<td>1.6</td>
<td>2.5E-05</td>
<td>0.03</td>
</tr>
<tr>
<td>CECR1</td>
<td>-1.4</td>
<td>4.2E-05</td>
<td>0.04</td>
<td>AGPAT9</td>
<td>1.5</td>
<td>3.3E-05</td>
<td>0.03</td>
</tr>
<tr>
<td>IGHD3-3</td>
<td>-2.2</td>
<td>4.4E-05</td>
<td>0.04</td>
<td>CARD6</td>
<td>1.5</td>
<td>4.7E-05</td>
<td>0.04</td>
</tr>
</tbody>
</table>

FC: Fold Change, FDR: False Discovery Rate
Table 3: Pathway analysis of the 100 most up and downregulated genes in samples from post-operative day 1 compared to pre-operative.

<table>
<thead>
<tr>
<th>Description of pathway</th>
<th># Genes in Overlap (k)</th>
<th>Unadjusted p-value</th>
<th>FDR q-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Down-regulated pathways:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Generation of second messenger molecules</td>
<td>8</td>
<td>$3.1 \times 10^{-16}$</td>
<td>$2.1 \times 10^{-13}$</td>
</tr>
<tr>
<td>Translocation of ZAP-70 to Immunological synapse</td>
<td>6</td>
<td>$1.3 \times 10^{-13}$</td>
<td>$3.1 \times 10^{-11}$</td>
</tr>
<tr>
<td>TCR signaling</td>
<td>8</td>
<td>$1.4 \times 10^{-13}$</td>
<td>$3.1 \times 10^{-11}$</td>
</tr>
<tr>
<td>Phosphorylation of CD3 and TCR zeta chains</td>
<td>6</td>
<td>$3.5 \times 10^{-13}$</td>
<td>$5.9 \times 10^{-11}$</td>
</tr>
<tr>
<td>PD-1 signaling</td>
<td>6</td>
<td>$8.1 \times 10^{-11}$</td>
<td>$1.1 \times 10^{-9}$</td>
</tr>
<tr>
<td>Downstream TCR signaling</td>
<td>6</td>
<td>$9.8 \times 10^{-10}$</td>
<td>$1.1 \times 10^{-8}$</td>
</tr>
<tr>
<td>Costimulation by the CD28 family</td>
<td>6</td>
<td>$2.8 \times 10^{-9}$</td>
<td>$2.7 \times 10^{-7}$</td>
</tr>
<tr>
<td>Immune System</td>
<td>14</td>
<td>$3.4 \times 10^{-9}$</td>
<td>$2.9 \times 10^{-7}$</td>
</tr>
<tr>
<td>Adaptive Immune System</td>
<td>11</td>
<td>$8.4 \times 10^{-9}$</td>
<td>$6.3 \times 10^{-7}$</td>
</tr>
<tr>
<td>Interferon gamma signaling</td>
<td>4</td>
<td>$7.2 \times 10^{-6}$</td>
<td>$4.8 \times 10^{-4}$</td>
</tr>
<tr>
<td><strong>Up-regulated pathways:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metabolism of carbohydrates</td>
<td>6</td>
<td>$1.7 \times 10^{-6}$</td>
<td>$1.1 \times 10^{-3}$</td>
</tr>
<tr>
<td>Glucose metabolism</td>
<td>4</td>
<td>$3.3 \times 10^{-6}$</td>
<td>$1.1 \times 10^{-3}$</td>
</tr>
</tbody>
</table>
Table 4: Fold change values for candidate genes in samples from post-operative day 1 compared to pre-operative

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function*</th>
<th>FC</th>
<th>Unadjusted p-value</th>
<th>FDR</th>
<th>ANOVA p-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8A</td>
<td>Alpha chain of TCR of CD8(^+) T-cells that binds to MHC-I molecules.</td>
<td>-1.3</td>
<td>0.0005</td>
<td>0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>CD4</td>
<td>CD4 TCR that binds MHCII on APC.</td>
<td>-1.6</td>
<td>7.7E-06</td>
<td>0.02</td>
<td>3.3E-07</td>
</tr>
<tr>
<td>CD3D</td>
<td>TCR on CD3(^+) T-cells consists of subunits from gene product of genes CD3D,E,G and CD247.</td>
<td>-1.7</td>
<td>9.9E-06</td>
<td>0.02</td>
<td>0.001</td>
</tr>
<tr>
<td>CD3E</td>
<td></td>
<td>-1.5</td>
<td>0.001</td>
<td>0.07</td>
<td>0.003</td>
</tr>
<tr>
<td>CD3G</td>
<td></td>
<td>-1.9</td>
<td>0.0001</td>
<td>0.04</td>
<td>0.002</td>
</tr>
<tr>
<td>CD247</td>
<td></td>
<td>-1.4</td>
<td>0.0010</td>
<td>0.06</td>
<td>0.01</td>
</tr>
<tr>
<td>GZMB</td>
<td>Granzyme B, cytotoxic molecule secreted by NK-cells and CD8(^+) T-cells.</td>
<td>-1.5</td>
<td>0.0006</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>HLA-A</td>
<td>MHC class I (\alpha) chain</td>
<td>-1.1</td>
<td>0.22</td>
<td>0.63</td>
<td>0.04</td>
</tr>
<tr>
<td>HLA-B</td>
<td>MHC class I (\beta) chain</td>
<td>-1.0</td>
<td>0.81</td>
<td>0.93</td>
<td>0.25</td>
</tr>
<tr>
<td>HLA-DRA</td>
<td>MHC class II HLA alpha chain</td>
<td>-1.6</td>
<td>0.008</td>
<td>0.15</td>
<td>0.004</td>
</tr>
<tr>
<td>Gene</td>
<td>Description</td>
<td>Log2 Fold Change</td>
<td>p-value</td>
<td>ngFDR</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------------------</td>
<td>---------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>HLA-DPB1</td>
<td>MHC class II HLA Beta chain paralogue.</td>
<td>-1.6</td>
<td>0.0002</td>
<td>0.04</td>
<td>0.0001</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Initiates multiple innate and cellular immune functions.</td>
<td>-1.1</td>
<td>0.26</td>
<td>0.66</td>
<td>0.38</td>
</tr>
<tr>
<td>EGF</td>
<td>Epidermal growth factor stimulates cell growth and differentiation.</td>
<td>1.1</td>
<td>0.28</td>
<td>0.67</td>
<td>0.003</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal growth factor receptor.</td>
<td>1.0</td>
<td>0.63</td>
<td>0.86</td>
<td>0.1</td>
</tr>
<tr>
<td>VEGFA</td>
<td>Increased vascular permeability, angiogenesis, endothelial cell growth, cell migration, and inhibiting apoptosis.</td>
<td>1.1</td>
<td>0.40</td>
<td>0.75</td>
<td>0.59</td>
</tr>
<tr>
<td>VEGFC</td>
<td>Increased vascular permeability, angiogenesis, endothelial cell growth, cell migration, and inhibiting apoptosis.</td>
<td>1.1</td>
<td>0.15</td>
<td>0.56</td>
<td>0.22</td>
</tr>
<tr>
<td>MMP9</td>
<td>Promotion of cell adhesion and migration.</td>
<td>2.7</td>
<td>2.7E-05</td>
<td>0.03</td>
<td>0.0003</td>
</tr>
<tr>
<td>CRP</td>
<td>Mediator of innate immunity.</td>
<td>1.0</td>
<td>0.45</td>
<td>0.77</td>
<td>0.12</td>
</tr>
<tr>
<td>IL1B</td>
<td>Mediator of innate immunity, cell proliferation, differentiation, and apoptosis.</td>
<td>1.5</td>
<td>0.002</td>
<td>0.08</td>
<td>0.01</td>
</tr>
<tr>
<td>Gene</td>
<td>Function Description</td>
<td>Pre-operative</td>
<td>Post-operative Day 1</td>
<td>Post-operative Day 2</td>
<td>Post-operative Day 3</td>
</tr>
<tr>
<td>------</td>
<td>----------------------</td>
<td>---------------</td>
<td>----------------------</td>
<td>----------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>IL6</td>
<td>Mediator of innate immunity, cell proliferation, differentiation, and apoptosis.</td>
<td>-1.0</td>
<td>0.97</td>
<td>0.99</td>
<td>0.05</td>
</tr>
<tr>
<td>TNF</td>
<td>Regulation of cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation.</td>
<td>1.0</td>
<td>0.98</td>
<td>0.99</td>
<td>0.46</td>
</tr>
<tr>
<td>IL10</td>
<td>Suppresses cell-mediated immunity</td>
<td>1.2</td>
<td>0.0004</td>
<td>0.04</td>
<td>0.0003</td>
</tr>
<tr>
<td>IL1RN</td>
<td>IL1 receptor antagonist</td>
<td>1.3</td>
<td>0.0003</td>
<td>0.04</td>
<td>0.001</td>
</tr>
<tr>
<td>TP53</td>
<td>Tumor suppressor gene, preventing genome mutation.</td>
<td>-1.4</td>
<td>4.6E-05</td>
<td>0.03</td>
<td>5.7E-05</td>
</tr>
</tbody>
</table>

* A short and non-exhaustive description of the function of the protein product encoded by the relevant gene.

** Differential gene expression analysis by repeated-measures ANOVA including results from all time-points (pre-operative, post-operative day 1, 2, 3 and 10-14).
Highlights

- Laparoscopic colon cancer causes dysregulation of immunologically relevant genes
- Downregulated immunological genes are overrepresented in pathway analysis.
- Genes relevant for antigen presentation, T-cell signaling and NK cytotoxicity is downregulated after surgery
- Gene transcription of inflammatory and immune-suppressive cytokines is upregulated after surgery