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Original research

Metabolism gene signatures and surgical site infections in abdominal surgery

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HIGHLIGHTS

- Surgical site infections (SSI) are related to both wound contamination with microorganisms and host susceptibility.
- We identified a unique gene expression signature in subcutaneous tissues of patients who developed SSI.
- Genes controlling cell metabolism were mostly down-regulated in subcutaneous tissues of those patients.
- This suggests increased inherent tissue susceptibility to infection due to locally reduced metabolic activity.

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ABSTRACT

Introduction: Surgical site infections (SSI) represent a significant cause of morbidity in abdominal surgery. The objective of this study was to determine the gene expression signature in subcutaneous tissues in relation to SSI.
Methods: To determine differences in gene expression, microarray analysis were performed from bulk tissue mRNA of subcutaneous tissues prospectively collected in 92 patients during open abdominal surgery. 10 patients (11%) developed incisional (superficial and deep) SSI.
Results: Preoperative risk factors in patients with SSI were not significantly different from those in patients without wound infections. 1025 genes were differentially expressed between the groups, of which the AZGP1 and ALDH1A3 genes were the highest down- and upregulated ones. Hierarchical clustering demonstrated strong similarity within the respective groups (SSI vs. no-SSI) indicating inter-group distinctness. In a functional classification, genes controlling cell metabolism were mostly down-regulated in subcutaneous tissues of patients that subsequently developed SSI.
Conclusion: Altered expression of metabolism genes in subcutaneous tissues might constitute a risk factor for postoperative abdominal SSI.

1. Introduction

Surgical site infections (SSI) are common complications of abdominal surgeries [1], significantly contributing to surgery-related morbidity and mortality and increasing the length of hospital stay and health-care costs [2,3]. Major risk factors are the estimated microbial contamination of the surgical site and host susceptibility. While standardized pre-operative antisepsis of the skin and antibiotic prophylaxis have markedly reduced the number of SSI [4,5], all surgical wounds are contaminated with microorganisms to a certain degree [6]. Thus, host susceptibility that varies between individuals, is an important factor that could be used to tailor prophylaxis strategies and to reduce SSI rates.

The wound healing process requires a coordinated activation of different cell types. Stem cells in hair follicles for example respond to wounds by generating a significant amount of proliferative

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keratinocytes that in turn contribute to the repair of the wounds [7–10]. Interestingly, the activity of hair follicle stem cells parallels the dynamic adipogenesis process and the presence of immature adipocytes is necessary and sufficient to activate these stem cells [11]. Furthermore, adipose-derived stem cells (ASCs) [12], which are of mesenchymal origin, are involved in multiple steps of wound healing either by providing substrate cells that participate directly in structural repair or by supplying secreted factors that sustain proliferation of various other cell types (e.g. fibroblasts and endothelial cells) [13,14]. In addition, specific cells in subcutaneous tissues (e.g. pre-adipocytes) display features of macrophages in that they can phagocytose and are capable of killing microorganisms [15–17]. Collectively, these data imply that both the healing ability and host defense are influenced by cells in the subcutaneous tissues. Indeed, it has been suggested that the individual composition, distribution, and the thickness of the subcutaneous tissue compartment influence susceptibility to SSI [18,19]. Here we determined the gene expression signature in subcutaneous tissues in relation to SSI in patients undergoing open abdominal surgeries.

2. Material and methods

2.1. Patients and tissue sampling

Subcutaneous adipose tissue was obtained from 92 patients (36 women, 56 men) during open abdominal surgery requiring a median or transverse laparotomy (small abdominal operations such as e.g. open appendectomies were excluded). Their age ranged from 24 to 96 years (mean ± SD: 62.7 ± 14.6 years). Subcutaneous adipose tissue was excised during laparotomy and all samples were frozen in liquid nitrogen and stored at −80 °C until RNA isolation. Wounds were evaluated daily by a surgeon during the postoperative course until discharge. Postoperative SSI were classified according to the CDC guidelines [5,20]. All patients gave written informed consent and the project was approved by the local Ethics committee of the Technische Universität München, Munich, Germany.

2.2. RNA isolation

Total subcutaneous tissue RNA was prepared using the RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Extracted RNA samples were stored at −80 °C until further use. RNA content was quantified by spectrophotometry. Concentrations per sample had to be higher than 100 ng/μl and absorption ratios at 260–280 nm and 260 to 230 nm had to be 1.8 or higher.

2.3. Patient data and matched pairs

Perioperatively, all patients received antibiotic prophylaxis; Skin preparation was carried out in a standardized fashion using povidone-iodine (Braunoderm®; B. Braun Melsungen AG, Germany); operating room conditions were identical for all patients. Postoperatively, each patient was observed for SSI at the surgical incisional site. Operation details such as the indication (neoplasia, infection), the contamination status of the operation, the operation site and the length of the procedure were documented. Patient data including physical characteristics (age, gender, BMI), medical histories (actual or past chemotherapy, steroid therapy, smoker status) and known medical illnesses (e.g. diabetes mellitus) were collected. For each SSI patient (only superficial and deep SSI; organ-space SSI were excluded), two patients with similar characteristics and medical histories with normal wound healing were matched. To this end, the highest priorities were given to diagnosis (infection vs. neoplasia) and wound classification (clean, clean-contaminated, contaminated [21]). Besides, age, BMI and the operation time were matched whenever possible. The basal metabolic rate (BMR) was calculated according to a revised Harris–Benedict Equation [22].

2.4. Microarray experiments

A whole transcriptome analysis of 30 subcutaneous tissue RNA samples (10 samples with SSI and 20 samples without SSI) was performed using the Affymetrix GeneChip® Human Gene 1.0 ST Array according to the manufacturer's instructions, as previously described [23].

2.5. Microarray analysis

Normalization was performed using the Robust Multichip Average (rma) method provided in the Bioconductor package of R (R Foundation for Statistical Computing, Vienna, Austria). Genes that were expressed at very low levels throughout all samples were filtered out using the filtering method nsFilter. All genes that were differentially expressed at p-values <0.05 were selected. A functional analysis to the group of the remaining genes was applied. Therefore, all GO (Gene Ontology) terms occurring among the set of genes were selected and a hypergeometric testing procedure to identify terms that were overrepresented in the set was performed. All terms that were overrepresented significantly ($p < 0.0001$, minimal category size $N = 100$) were selected and all genes that were differentially expressed and annotated by at least one of the overrepresented GO terms were visualized in a heatmap. The arrangement of the samples in the heatmap was performed with an unsupervised clustering by the application of the hclust function in R, using a complete linkage distance function.

2.6. Identification of expression patterns

In order to identify combinations of a small number of differentially expressed genes that characterize the two groups with a high accuracy, the concept of expression patterns is introduced by the following described method. An *expression pattern* $f(g_1, \ldots, g_n)$ is a boolean function containing NOT and AND operators only. Each symbol in the expression represents one gene, e.g. $\int(A, B, C) = A\ AND\ NOT\ B\ AND\ C$. An expression pattern is called *fulfilled* for a given assignment of its variables (genes) $g_1, \ldots, g_n$ if $\int g_1, \ldots, g_n) = 1$. The expression values of all genes ($g_{i,s}$), i indexing genes and s indexing samples, were discretized for all samples by thresholding at the mean $\mu(g_i)$, i.e. by assigning $b_{i,s} = \begin{cases} 1\ if\ g_{i,s} \geq \mu(g_i) \\ 0\ else \end{cases}$. The discretized expression values were used to check whether a given expression pattern f is fulfilled for some samples by evaluating $f(b_{h1,s}, \ldots b_{hn,s}) = 1$ where $h_1, \ldots, h_n$ are the indexes of the genes that are input variables. In order to find expression patterns that are highly specific and sensitive to the groups (SSI, match group), all possible expression patterns containing up to 6 input variables were considered, where each variable was chosen from the top-50 differentially expressed genes, i.e. the total number of possible patterns is $\sum_{i=1}^{6} \binom{50}{i} 2^i \approx 1.1 \cdot 10^9$. The results showed that using more than 2 input variables did not improve the accuracy of the expression patterns, thus, patterns with 2 input variables were chosen. For a given expression pattern f the probability to observe an SSI sample under the condition that the rule is fulfilled was computed by $P(SSI \mid f = 1) = \frac{P(f=1|SSI)P(SSI)}{p(f=1)}$. Rules were selected only if the probability was 1 which implies that the specificity of the rule is 1. The sensitivity was then computed as