A “death signature” in patients with head and neck cancer.

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Differential gene expression analysis has the potential to illuminate fundamentals of biology by identifying key differences in transcription between cells, genotypes or diseases. I compared the expression profiles of 327 genes in a cohort of patients with head and neck cancer (1), grouped based on their survival at three years. Patients who were dead at three years possessed a unique “death signature” of forty genes whose expression was significantly different from that of survivors. This gene expression signature, associated with more rapid mortality in humans with head and neck cancer, may have clinical relevance in prognostic stratification and the genes themselves may have biological significance in the etiology of head and neck cancer.

Keywords: head and neck cancer, differential gene expression

Introduction

Differential gene expression analysis possesses limitless possibilities for the elucidation of the major transcription differences between any number of groups greater than one. For example, by comparing gene expression in cells deficient or wild-type for a molecule, the function of a molecule can be ascertained by studying the transcripts that are most different between these groups. Another example is the study of patients with and without a disease, or the analysis of cells that have been treated with a drug or not. Previously, I identified major transcriptional differences in patients with sepsis and systemic inflammatory syndrome, and in patients with T-cell acute lymphoblastic leukemia (T-ALL) bearing either the gamma-delta or alpha-beta T-cell receptor using differential gene expression analysis (2,3).

Here, I performed differential gene expression analysis of tumors of patients with head and neck cancer based on their survival at three years (1). I utilized a gene expression dataset (327 genes involved in immunity and cancer (1)) from a cohort of thirty-six patients with head and neck cancer, twenty of whom were alive at three years and sixteen who were not. I identified a group of forty genes whose expression was significantly different between patients alive or dead at thirty-six months. Moreover, I found that the gene expression of AIRE, a molecule involved in immunological tolerance, trended towards being significantly different between patients with head and neck cancer alive or dead at three years.

Methods

Differential gene expression analysis of dataset GSE122272 was performed using GEO2R (1). This identified the two hundred and fifty most differentially expressed genes in tumors between survivors and non-survivors at three years based on a nCounter gene expression panel of immune and cancer genes designed by the authors of the study that produced the data analyzed here (1). No adjustment was made to p-values, and data was not log-transformed. All genes identified here were determined to be statistically significantly differentially expressed by NCBI. Additional statistical test to compare the difference between

the actual RNA expression values of each gene between survivors and non-survivors was performed by an unpaired t-test with Welch's correction (PRISM 8.1.2).

Results

I performed differential gene expression analysis on tumors from survivors and non-survivors of head and neck cancer at three years using a previously published dataset of thirty-six patients with head and neck cancer, twenty of whom were alive at 3 years and sixteen of whom were dead (1). I identified a panel of 40 genes whose expression in tumors was significantly different between three year survivors and non-survivors (Figure 1).

When comparing the expression of each of 327 genes between survivors and non-survivors at three years, STAT3 was the gene most differentially expressed by three year non-survivors in head and neck cancer. STAT3 expression was significantly higher among patients dead at 3 years (Figure 2; $p=0.0063$).

DDX43 was the second most differentially expressed gene by three year non-survivors in head and neck cancer. The expression of Ddx43 was significantly higher in patients who were dead at 3 years (Figure 3; $p=0.0125$).

Multiple soluble immune factors were differentially expressed by patients who had succumbed to disease at three years. IL15 and IL34 were both expressed at higher levels by patients dead at three years (Figure 4; $p=0.0133$ and $p=0.0117$, respectively). CX3CL1 and LTA were also expressed at significantly higher levels by three year non-survivors (Figure 4; $p=0.0271$ and $p=0.0294$, respectively).

Non-survivors differentially expressed two chemokine receptors. CXCR4 and CXCR6 were expressed at significantly higher levels by three year non-survivors (Figure 5; $p=0.0398$ and $p=0.0244$, respectively).

Cell adhesion molecules ALCAM, NCAM1 and TICAM2 were differentially expressed by three year non-survivors. Interestingly, ALCAM, NCAM1 and TICAM2 were all expressed at significantly lower levels in patients who had succumbed to disease at three years (Figure 6; $p=0.0163$, $p=0.0167$ and $p=0.0201$, respectively).

Genes encoding cluster of differentiation markers were also differentially expressed by patients succumbing to disease at three years. CD7, CD83, CD164, CD40 and CD200 were all expressed at significantly higher levels by patients with head and neck cancer who had succumbed by three years (Figure 7; $p=0.0122$, $p=0.0227$, $p=0.0175$, $p=0.0283$, and $p=0.0458$, respectively).

Transcription factors ETS1, TAL1 and ATF2 were differentially expressed by three year non-survivors. ETS1, TAL1 and ATF2 were all expressed at significantly higher levels by three-year non-survivors (Figure 8; $p=0.0222$, $p=0.0319$, and $p=0.0265$, respectively).

Human leukocyte antigen genes HLA-DMB and HLA-DPA1 were differentially expressed by three year non-survivors. HLA-DMB and HLA-DPA1 were both expressed at significantly higher levels in patients who had succumbed to disease by three years (Figure 9; $p=0.0137$ and $p=0.0281$, respectively).

Apoptotic pathway genes BAX and BCL2 were differentially expressed by three year non-survivors. BAX was expressed at significantly lower levels in non-surviving patients (Figure 10; $p=0.0242$) while BCL2 was expressed at significantly higher levels in non-surviving patients (Figure 10; $p=0.0432$).

Two interferon-stimulated genes, ISG20 and IFI35 were differentially expressed by three year non-survivors. ISG20 and IFI35 were expressed at significantly higher levels by non-survivors (Figure 11; $p=0.0398$ and $p=0.0314$, respectively).

Interestingly, AIRE was differentially expressed by three year non-survivors in head and neck cancer. AIRE plays an important role in the expression of tissue-restricted antigens in the thymus (4, 5). AIRE was expressed at higher levels by non-survivors, and this trended towards statistical significance (Figure 12; $p=0.0614$).

The expression of fifteen other genes, not otherwise classified, was significantly different between patients with head and neck cancer succumbing or surviving by three years (Figure 13). These included ATG10 ($p=0.0077$), LAG3 ($p=0.0123$), TPSAB1 ($p=0.0165$), PIK3CD ($p=0.0194$), G6PD ($p=0.0087$), DPP4 ($p=0.0225$), Ly96 ($p=0.0180$), KLRC1 ($p=0.0365$), TFRC

($p=0.0185$), IKBKG ($p=0.0266$), MR1 ($p=0.0415$), PSEN2 ($p=0.0385$), OAS2 ($p=0.0452$), TLK2 ($p=0.0326$), and PSMB9 ($p=0.0362$) (Figure 13). The expression of all of these genes was higher in non-survivors, except for G6PD, IKBKG, and TFRC which were expressed at lower levels in non-survivors (Figure 13).

Discussion

I compared the expression of 327 genes between patients with head and neck cancer who were dead or alive at three years (1). Patients dead at three years possessed a unique gene expression signature composed of a group 40 genes. These included genes encoding chemokine receptors, cell adhesion molecules, interleukins, and transcription factors, among others.

Genes that are differentially expressed by non-survivors could be important in different ways. These genes could have significance in the etiology of the disease, leading to a more aggressive course of disease. Another possibility is that the expression of these genes is a reflection of the more aggressive nature of the disease rather than a manifestation of the underlying role for these genes in the pathogenesis of the disease itself. Future work to evaluate the role of any of these genes in transformation or maintenance of the transformed state in head and neck cancer is necessary to validate the significance of any of these genes in the diagnosis and treatment of head and neck cancer.

Nevertheless, the differential expression of the forty genes identified here among non-survivors provides an immediate tool by which the gene expression of patients with head and neck cancer may be leveraged to prospectively identify patients who may benefit from more aggressive treatment.

References

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Figure 1: A “death signature” in head and neck cancer.

The exact expression value of all forty indicated genes in the tumors of patients who have survived (n=20) or not survived (n=16) head and neck cancer at three years is shown.

Figure 2: Stat3 is differentially expressed in patients succumbing to head and neck cancer by 3 years.

The expression value of the indicated gene in patients who have survived (n=20) or not survived (n=16) head and neck cancer at three years is shown. The bar represents the mean expression of the gene in the group of patients, and each dot represents the expression of that gene in an individual patient.

Figure 4: Soluble immune factors are differentially expressed in patients succumbing to head and neck cancer by 3 years.

The expression value of the indicated gene in patients who have survived (n=20) or not survived (n=16) head and neck cancer at three years is shown. The bar represents the mean expression of the gene in the group of patients, and each dot represents the expression of that gene in an individual patient.

Figure 5: Chemokine receptors are differentially expressed in patients succumbing to head and neck cancer by 3 years.

The expression value of the indicated gene in patients who have survived (n=20) or not survived (n=16) head and neck cancer at three years is shown. The bar represents the mean expression of the gene in the group of patients, and each dot represents the expression of that gene in an individual patient.

Figure 6: Cell adhesion molecules are differentially expressed in patients succumbing to head and neck cancer by 3 years.

The expression value of the indicated gene in patients who have survived (n=20) or not survived (n=16) head and neck cancer at three years is shown. The bar represents the mean expression of the gene in the group of patients, and each dot represents the expression of that gene in an individual patient.

Figure 7: CD7, CD83, CD164, CD40 and CD200 are differentially expressed in patients succumbing to head and neck cancer by 3 years.

The expression value of the indicated gene in patients who have survived (n=20) or not survived (n=16) head and neck cancer at three years is shown. The bar represents the mean expression of the gene in the group of patients, and each dot represents the expression of that gene in an individual patient.

Figure 8: Transcription factors are differentially expressed in patients succumbing to head and neck cancer by 3 years.

The expression value of the indicated gene in patients who have survived (n=20) or not survived (n=16) head and neck cancer at three years is shown. The bar represents the mean expression of the gene in the group of patients, and each dot represents the expression of that gene in an individual patient.

Figure 9: HLA-DMB and HLA-DPA1 are differentially expressed in patients succumbing to head and neck cancer by 3 years.

The expression value of the indicated gene in patients who have survived (n=20) or not survived (n=16) head and neck cancer at three years is shown. The bar represents the mean expression of the gene in the group of patients, and each dot represents the expression of that gene in an individual patient.

Figure 10: Bax and Bcl2 are differentially expressed in patients succumbing to head and neck cancer by 3 years.

The expression value of the indicated gene in patients who have survived (n=20) or not survived (n=16) head and neck cancer at three years is shown. The bar represents the mean expression of the gene in the group of patients, and each dot represents the expression of that gene in an individual patient.

Figure 11: *Isg20* and *Ifi35* are differentially expressed in patients succumbing to head and neck cancer by 3 years.

The expression value of the indicated gene in patients who have survived (n=20) or not survived (n=16) head and neck cancer at three years is shown. The bar represents the mean expression of the gene in the group of patients, and each dot represents the expression of that gene in an individual patient.

Figure 13: Other genes differentially expressed in patients succumbing to head and neck cancer by 3 years.

The expression value of the indicated genes in patients who have survived (n=20) or not survived (n=16) head and neck cancer at three years is shown. The bar represents the mean expression of the gene in the group of patients, and each dot represents the expression of that gene in an individual patient.