Gene Expression Profiling in Cervical Cancer: An Exploration of Intratumor Heterogeneity

Barbara Bachtiary,1,4,9 Paul C. Boutros,3,5,6 Melania Pintilio,2 Willa Shi,4 Carlo Bastianutto,4 Jian-Hua Li,4 Joerg Schwock,4,7,8 Wendy Zhang,2,6 Linda Z. Penn,3,6 Igor Jurisica,2,5,6 Anthony Fyles,1 and Fei-Fei Liu4,6

Abstract

Purpose: To explore intratumor heterogeneity in gene expression profiles from patients with cervical cancer.

Experimental Design: A total of 33 biopsies were obtained from 11 patients, sampling between two and five different areas for each tumor. The extracted RNA was hybridized onto the Affymetrix U133 Plus 2.0 oligonucleotide chip. The variance of expression within a patient (W), between patients (B) and the total variance (T = W + B) were calculated for each ProbeSet, and the ratio W/T was used as a measure of intratumor heterogeneity. Gene Ontology functional analysis was done to assess the function of genes that had high W/T (top 10%) and low W/T (bottom 10%) values.

Results: In total, 448 ProbeSets (2.2% of the total) had W/T < 0.10, indicating low intratumor heterogeneity, and 537 ProbeSets (2.7% of the total) had W/T > 0.90, indicating high intratumor heterogeneity. In total, 14,473 ProbeSets (72.4%) had higher intratumor than intratumor heterogeneity (W/T < 0.5). Genes with low intratumor heterogeneity were characterized by a statistically significant enrichment of immune-related functions (P < 0.0001). Genes with high intratumor heterogeneity were characterized by a significant tendency towards nuclear localization and nucleic acid binding (both P < 0.0001). For genes with W/T > 0.5, more than six biopsies would be required to minimize the intratumoral heterogeneity to <0.15; if W/T is 0.3 to 0.4, four biopsies are required; and for low W/T of 0.16 to 0.3, only two to three biopsies would be needed.

Conclusion: Although the intratumor heterogeneity was low for the majority of the tested ProbeSets, for many genes, multiple biopsies are required to obtain a reliable estimate of gene expression.

Worldwide, cervical cancer is the second most common cancer in women. The IARC estimated 493,000 new cases and 274,000 deaths worldwide in the year 2003 (1). For patients with locally advanced disease, the standard treatment is radiotherapy. The 5-year survival rate ranges from 80% in stage IB2 disease to <50% in stage IIIB tumors. The most important prognostic factor for survival is tumor size (2); however, tumors vary considerably in their biological behavior, and some small cancers develop distant metastases, whereas some bulky tumors remain localized to the cervix. The identification of specific molecular patterns or markers that could predict the individual biological behavior of a tumor would help to further classify cervical cancers. Patients with more aggressive tumors could then be identified early and treated with individually targeted therapies. Thus far, no generally accepted molecular marker for cervical cancer has been reported.

In order to discover specific differentially expressed prognostic and/or predictive molecular markers, several studies have analyzed transcript expression in cervical cancers using microarray technologies (3–8). Interestingly, panels of molecular markers, which have been found to be differently expressed between normal and cancer tissues, or to be predictive for response to therapy, lack consistency in the published literature. To some extent, this may be explained by the use of different study designs and microarray platforms, and because investigating thousands of genes means that many different but equally prognostic/predictive signatures could be derived (9). Another reason for the diverging study outcomes, however, may be intratumor heterogeneity in mRNA and protein expression (10).
Intratumor heterogeneity is a recognized characteristic for human cervical cancer, and occurs on multiple levels. At the genetic level, DNA-ploidy, chromosomal aberrations, and mutations in specific genes vary considerably within any one individual tumor (11–17). At the protein level, intratumor heterogeneity in the expression of specific proteins is often described in immunohistochemical studies (18, 19). Finally, at the macroscopic level, blood perfusion, oxygen pressure, and interstitial fluid pressures differ significantly from region to region within an individual tumor (20–23). The logical extrapolation of these variations would predict that there would be significant intratumoral heterogeneity of gene expression profiles for human cervical carcinoma. However, to date, no such data are yet available.

The aim of this current study therefore, is to fill this gap, using a preliminary exploration of the extent of intratumoral heterogeneity of gene expression in human cervical cancer. Through this process, we developed a novel statistical metric for characterizing the intratumoral heterogeneity in gene expression and used this measure to functionally group genes with low or high intratumor heterogeneity. Finally, we modeled and provide an estimate on the number of samples required in order to minimize heterogeneity for a specific group of genes.

**Materials and Methods**

**Specimen collection.** Sixty-six flash-frozen punch biopsies were obtained from 16 patients with cervical cancer who were undergoing examination under general anesthesia as part of their pretreatment evaluation for cervical cancer. From each patient, four to seven biopsies were obtained from different regions of the tumor. Immediately upon removal from the patient, the specimens were bisected; one-half was placed in a storage medium (optimal cutting temperature compound) for conventional histopathologic examination, then immediately flash-frozen in liquid nitrogen. The other half of each sample was flash-frozen without any storage medium. The specimens were subsequently evaluated by a pathologist (I. Schwock) based on the morphology of the frozen H&E-stained tissue sections cut from the optimal cutting temperature–embedded material. The total cell content—including stroma and tumor cells—was estimated for all 66 tissue samples using a light microscope. Only samples which contained >50% tumor cells were considered for further analysis. According to this, 33 tissue samples from 11 different patients were included with a range of tumor cell fractions of 60% to 100% (Supplementary Table S1; Supplementary Fig. S1).

Pretreatment evaluation for these patients included bimanual rectovaginal palpation of the tumor, intratumor oxygenation, and interstitial fluid pressure measurements as previously described (Supplementary Table S1; Supplementary Fig. S1).

**RNA purification.** Total RNA was purified using the Absolutely RNA Microprep Kit (Stratagene, La Jolla, CA). Briefly, tumor tissue was lysed in guanidine thiocyanate, mixed with ethanol, and RNA was captured on a silica-based fiber matrix within a microspin cup. RNA was then washed with saline buffers, DNA contamination was removed with DNase, and RNA was then eluted in RNase-free water. The quality of the purified RNA was assessed by analyzing 200 pg of each sample using a Bioanalyzer 2100 (Agilent, Palo Alto, CA). Only samples with a 28S/18S ribosomal peak ratio of 1.8 to 2.0 were considered suitable for labeling.

**Microarray hybridization.** The Human Genome U133A Plus 2.0 Gene Chip (Affymetrix, Santa Clara, CA), which contains 54,675 ProbeSets, representing 24,325 distinct UniGene clusters, was used for this study. A total of 1.5 μg of purified total RNA template was reverse-transcribed to generate double-stranded cDNA. Following second-strand cDNA synthesis, biotin-labeled antisense cRNA was generated by in vitro transcription. Next, 15 μg of each generated cRNA preparation was fragmented and hybridized to an oligonucleotide array. Automated washing, staining, and scanning was done according to the manufacturer’s protocols.

**Normalization and analysis.** The raw data were preprocessed using the GCRMA algorithm (26). First, the expression signals of the perfect match probes were corrected for optical noise and nonspecific binding by incorporating mismatch probe information. Next, individual probe intensities were smoothed through quantile normalization (27). Finally, expression values for each ProbeSet were generated using a median polish (28). This algorithm was implemented in the GCRMA package (v1.1.3) of the Bioconductor open source library (29) for R (version 2.0.1). Raw and normalized microarray data have been deposited in the GEO repository at NCBI under accession GSE5787.

**Filtering process and clustering.** The resulting expression values were filtered to remove low-intensity signals from unexpressed genes, which represents experimental noise. Because only samples from female patients were analyzed, signals of Y chromosome genes should reflect nonspecific hybridization. Therefore, Y chromosome genes were used to estimate a threshold that defines whether or not a gene is expressed. Accordingly, 34,675 ProbeSets with GCRMA-normalized signal intensities <4.0 were excluded (Supplementary Fig. S2). Unless noted otherwise, all additional analyses employed only the 20,000 ProbeSets with normalized intensities >4.0. Unsupervised hierarchical agglomerative clustering was done using the cluster package in R software (version 2.0.1) and the Euclidian distance as a measure of dissimilarity.

**ANOVA.** A variance-component analysis of the expression values for the 20,000 putatively expressed ProbeSets was done. The variance within a patient ($W = \text{variance due to differences within a tumor}$), the variance between patients ($B = \text{variance due to differences between patients}$) and the total variance ($T = W + B$) were produced. The ratio $W/T$ was then calculated and used as a measure of intratumor heterogeneity.

**Table 1. Patients and tumor characteristics**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients, n (%)</th>
<th>Number of biopsies, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Median 47</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Range 30-70</td>
<td></td>
</tr>
<tr>
<td>Federation Internationale des Gynaecologistes et Obstetristes stage</td>
<td>IB 2 (18)</td>
<td>6 (18)</td>
</tr>
<tr>
<td></td>
<td>IIB 4 (36)</td>
<td>15 (46)</td>
</tr>
<tr>
<td></td>
<td>IIIB 4 (36)</td>
<td>10 (30)</td>
</tr>
<tr>
<td></td>
<td>IVB 1 (9)</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td>≤5 4 (36)</td>
<td>13 (39)</td>
</tr>
<tr>
<td></td>
<td>&gt;5 7 (64)</td>
<td>20 (61)</td>
</tr>
<tr>
<td>Histology</td>
<td>Squamous 9 (82)</td>
<td>29 (88)</td>
</tr>
<tr>
<td></td>
<td>Adenocarcinoma 2 (18)</td>
<td>4 (12)</td>
</tr>
<tr>
<td>Differentiation</td>
<td>Well 1 (9)</td>
<td>2 (2)</td>
</tr>
<tr>
<td></td>
<td>Moderate 7 (64)</td>
<td>21 (70)</td>
</tr>
<tr>
<td></td>
<td>Poor 2 (18)</td>
<td>7 (23)</td>
</tr>
<tr>
<td></td>
<td>Unknown 1 (9)</td>
<td>3 (5)</td>
</tr>
<tr>
<td>Hemoglobin at diagnosis (g/dL)</td>
<td>&lt;12.6 4 (36)</td>
<td>10 (30)</td>
</tr>
<tr>
<td></td>
<td>&gt;12.6 7 (64)</td>
<td>23 (70)</td>
</tr>
<tr>
<td>Pelvic nodes</td>
<td>Positive 4 (36)</td>
<td>12 (36)</td>
</tr>
<tr>
<td></td>
<td>Equivocal 1 (9)</td>
<td>3 (9)</td>
</tr>
<tr>
<td></td>
<td>Negative 6 (55)</td>
<td>18 (55)</td>
</tr>
</tbody>
</table>
20,000 ProbeSets was ranked according to their functional enrichment in each decile relative to the entire set of 20,000 ProbeSets. The identified categories were grouped manually and color-coded according to the percentage of all annotated ProbeSets in any given decile.

Real-time quantitative PCR analysis. Real-time PCR (RT-PCR) amplification was done on 30 of the 33 samples, using primer sets for ACTB (as a normalization gene), DUSP1, CD55, IRAK1, CSTA, IL8, HIF-1α, and VEGF genes. Three samples were excluded due to insufficient material. β-Actin was used as a control for the amounts of cDNA generated from each sample. Synthesis of the first-strand cDNA was carried out using SuperScript First-Strand Synthesis System for QRT-PCR (Invitrogen, Carlsbad, CA). The RT product (1-3 μL) was then amplified for 40 cycles (2 minutes at 50°C, 15 minutes at 95°C, 15 seconds at 94°C, 30 seconds at 58°C) followed by an extension of 30 seconds at 72°C. Each assay was repeated thrice, and the mean ΔCT values were used for further calculations. The sequences of the PCR primer pairs were aligned to the mRNA, and to the Affymetrix ProbeSet for these genes (Supplementary Table S2). A Spearman correlation analysis for signal intensity of the microarray data and ΔC_{T} values of quantitative RT (QRT)-PCR data was then done.

Estimation of the number of biopsies required as a function of gene expression heterogeneity. The total variance was divided between variance due to patient heterogeneity (σ_{p}^{2}) and variance due to tumor heterogeneity (σ_{c}^{2}). When more than one sample per patient is analyzed, the variance of the mean value per patient decreases as the number of replicates per patient increases. Thus, the variance of the mean per tumor when 6 samples are analyzed is: W = σ_{p}^{2}/6 + σ_{c}^{2}/6. The total variance in this case is: \[ T = \sigma_{p}^{2} + W = \frac{\sigma_{p}^{2}}{6} + \frac{\sigma_{c}^{2}}{6}. \]

Results

Details of patient and tumor characteristics are provided in Table 1, describing features typical of cervical cancer patients.

Table 2. Genes selected for RT-PCR

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene title</th>
<th>Gene ID</th>
<th>Probe Set ID</th>
<th>Chromosomal location</th>
<th>W/T Microarray</th>
<th>W/T QRT-PCR</th>
<th>Correlation coefficient (ρ)*</th>
<th>Correlation coefficient (ρ)* after exclusion of two outlier samples</th>
<th>P value after exclusion of two outlier samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAF</td>
<td>Decay acceleration factor for complement</td>
<td>1604</td>
<td>1555950_a_at</td>
<td>Chr1q32</td>
<td>0.05</td>
<td>0.28</td>
<td>0.821</td>
<td>0.828</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CSTA</td>
<td>Cystadenin A</td>
<td>1475 3654</td>
<td>204971_at 201587_s_at</td>
<td>Chr3q21 ChrXq28</td>
<td>0.07</td>
<td>0.40</td>
<td>0.586</td>
<td>0.669</td>
<td>0.0007</td>
</tr>
<tr>
<td>IRAK1</td>
<td>Interleukin-1 receptor-associated kinase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DUSP1</td>
<td>Dual specificity phosphatase 1</td>
<td>1843</td>
<td>201041_s_at</td>
<td>Chr5q34</td>
<td>0.81</td>
<td>0.78</td>
<td>0.658</td>
<td>0.717</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HIF-1α</td>
<td>Hypoxia-inducible factor 1α subunit</td>
<td>3091</td>
<td>200989_at</td>
<td>Chr14q21-q24</td>
<td>0.19</td>
<td>0.83</td>
<td>0.255</td>
<td>0.376</td>
<td>0.18</td>
</tr>
<tr>
<td>IL8</td>
<td>Interleukin 8</td>
<td>3576 7422</td>
<td>202859_x_at 210512_s_at</td>
<td>Chr4q14-q24 Chr6p12</td>
<td>0.60</td>
<td>0.61</td>
<td>0.833</td>
<td>0.882</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelium growth factor</td>
<td></td>
<td></td>
<td></td>
<td>0.70</td>
<td>0.27</td>
<td>0.743</td>
<td>0.770</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Correlations between the microarray and QRT-PCR measurements.
who are treated with chemoradiotherapy. On average, three biopsies were obtained from different regions of the tumor from each patient.

**Variance analysis.** The ratio $W/T$, which is a measure of the intratumor heterogeneity in gene expression, was calculated for each of the 20,000 putatively expressed ProbeSets. Approximately equal numbers of ProbeSets displayed low intratumor heterogeneity ($W/T < 0.1$; 448 ProbeSets (2.2%)) and high intratumor heterogeneity ($W/T > 0.9$; 537 ProbeSets (2.7%)). The majority (95%) of ProbeSets fell between these two extremes, but with a global tendency towards lower intratumor heterogeneity. In total 14,473 ProbeSets (72.4%) had higher intertumor than intratumor heterogeneities ($W/T < 0.5$; Fig. 1); thus, for most genes, there was a greater variation between patients' tumors than within any single individual's tumor.

In order to explore whether the intratumor heterogeneity is dependent on the expression level of a gene, we examined the relationship between ProbeSet signal intensity and $W/T$. The $W/T$ values were dichotomized according to the mean ProbeSet signals. A significant trend was observed with higher mean signal intensities being represented by genes with lower $W/T$ ratios ($P < 0.0001$, $\chi^2$ test; Supplementary Table S3), suggesting that genes with higher expression levels are associated with reduced intratumor heterogeneity. However, it must be stressed that the very small $P$ value is mainly due to the very large number of genes analyzed.

**Validation with QRT-PCR.** In order to internally validate the results obtained from the microarray data, we selected seven representative genes with different $W/T$ ratios for further evaluation (Table 2). Three genes were selected which had

![Fig. 2. Scatter plots demonstrating intratumoral and intertumoral variability for selected genes. Scatter plots of signal intensities of ProbeSets representing genes with $W/T < 0.15$ (DAF, CSTA, and IRAK1), with $W/T > 0.75$ (DUSP1) and genes related to angiogenesis (HIF-1α, IL8, and VEGF). The $x$-axis denotes the individual patients ($n = 11$); each dot indicates the expression intensity of that gene, from each sample obtained from the corresponding patient ($n = 33$). The $y$-axis represents normalized signal intensity. Dots that cluster together show minimal intratumor variation (e.g., DAF); dots that are further apart show a higher degree of intratumor variation (e.g., DUSP1).](https://www.aacrjournals.org/doi/10.1158/1078-0432.CCR-06-0786)
low intratumor heterogeneity (low W/T values), included DAF, CSTA, and IRAK1. One gene with high intratumor heterogeneity (high W/T ratio) was chosen (DUSP1), and three genes functionally linked to hypoxia and angiogenesis were also selected (HIF-1α, IL8, and VEGF). The variation of each of these genes within and between patients is illustrated graphically in Fig. 2, indicating that genes with low intratumor heterogeneity (low W/T ratios) indeed do cluster closely within each patient's tumor, whereas DUSP1 (with high W/T) has different degrees of expression intensities within many patients' tumors.

Table 2 provides further details on each of these genes, including their putative function, and chromosomal locations. The W/T ratios are also provided for both the microarray signal intensity, and the QRT-PCR ΔCt values, along with correlations between the microarray and QRT-PCR measurements in terms of the W/T values. A strong correlation (correlation coefficient > 0.7) was observed for DAF, IL8, and VEGF; satisfactory correlations (0.5-0.7) were observed for CSTA, IRAK1, and DUSP1. No correlation was observed, however, for HIF-1α.

Two samples were identified as “outliers” in all QRT-PCR results (Fig. 3). When these samples were re-examined in greater detail, we went back to the original QRT-PCR data, samples labeled “12.5” and “4.1” gave unreliable results, indicating that RNA may have been damaged after repeated thawing and freezing. After excluding those two samples, the correlation coefficients were 0.828 for DAF, 0.834 for IRAK1, 0.770 for VEGF, 0.717 for DUSP1, 0.822 for IL8, and 0.669 for...
shown for ProbeSets whose expression heterogeneity is limited to W/T related to its regulatory heterogeneity, we attempted to correlate if the intratumoral heterogeneity in gene expression might be putatively expressed ProbeSets). The Spearman correlation analysis was repeated, but restricted to ProbeSets with low heterogeneity (<0.5 (B)). Each branch of the tree represents an individual sample from each patient (samples are labeled: "s," patient ID, underscore, and sample number for that patient). All samples, except one, clustered perfectly with the corresponding patient, indicating greater similarity in gene expression pattern within patients, than between different patients (A). This is even more clearly shown for ProbeSets whose expression heterogeneity is limited to W/T values of 0.5 (B).

CSTA, indicating a very good correlation between microarray and QRT-PCR results (Table 2; Fig. 3).

Hierarchical clustering. An unsupervised clustering was done to determine the extent of similarity in global gene expression pattern for each patient’s tumor, and whether different patients are distinguishable on the basis of this profile. When a cluster analysis was done on all 33 samples, all but one specimen (s10_7) clustered perfectly within each corresponding patient, indicating that the expression pattern is very similar within each patient (Fig. 4A). This clustering suggests that there is a unique expression profile that characterizes each patient’s tumor. Furthermore, when the cluster analysis was repeated, but restricted to ProbeSets with low intratumoral heterogeneity (W/T < 0.5), this resulted in perfect clustering of samples within each corresponding patient (Fig. 4B).

Prediction of expression heterogeneity. In order to determine if the intratumoral heterogeneity in gene expression might be related to its regulatory heterogeneity, we attempted to correlate the W/T values determined in our study with estimates of intertissue variability obtained from the GNF Gene Atlas (30). We selected only those ProbeSets present identically on the array platforms used for both studies (12,533 of the 20,000 putatively expressed ProbeSets). The Spearman correlation coefficients between these measures are shown in Supplementary Table S4. Only very weak correlations were observed, suggesting that intertissue heterogeneity does not account for the variations in gene expression intensity within each tumor.

Functional analysis of low versus high W/T genes. The 20,000 ProbeSets in our data set represent 11,141 distinct genes, based on the Affymetrix annotation. We wished to determine if intratumor gene expression heterogeneity might be related to distinct biological functions, hence, we tested the relationship between W/T values as a function of Gene Ontology (GO) annotations, using the GOMiner software. A total of 61 GO categories seem to be enriched for genes with very high intratumor homogeneity (P < 0.001 in the either of the two lowest deciles), or with high intertumor heterogeneity (P < 0.001 in either of the two highest deciles). These categories were grouped manually according to functionally related groups (see Fig. 5).

Transcripts with low W/T (<10%) were characterized by a statistically significant enrichment of genes with immune-related function, i.e., antigen processing and MHC class II receptor function (P < 0.0001; Fig. 5). Genes with high W/T were characterized by a significant tendency towards nuclear localization and nucleic acid binding (both P < 0.0001). Fully 29% (85 of 293) of genes with W/T > 0.95 were involved in nuclear localization, in comparison with only 19% (2,148 of 11,141) of genes in the complete data set (Fig. 5).

Estimation of the number of biopsies required as a function of gene expression heterogeneity. A model to estimate the necessary number of biopsies required to obtain reliable expression results for each gene based on the W/T of that gene, was developed (Fig. 6; refs. 32, 33). Using this model, the number of biopsies and the corresponding reduction in W/T were estimated. A stringent measure to reduce heterogeneity would be W/T ≤ 0.15, shown as black circles in Fig. 6 (32, 33). The model predicts that as the W/T value for a gene increases (greater intratumoral heterogeneity), a larger number of biopsies would be necessary to obtain a representative “whole tumor” expression for that specific gene. Hence, for genes with a W/T ≤ 0.15, a single biopsy would suffice; for genes with W/T ranging from 0.16 to 0.25, two biopsies are necessary. For genes with W/T values between 0.26 and 0.30, three biopsies would be required; for genes with W/T 0.3 to 0.4, four biopsies would be necessary. Finally, for highly heterogeneously expressed genes with W/T values >0.5, six or more biopsies would be required.

Discussion

The advent of high-throughput technologies has enabled the definition of patients’ tumors as a function of their gene expression profile (34). One concerning aspect of the literature is that different studies report different sets of potentially prognosticating genes, with little overlap among these reports (7, 8). There are many reasons for this variation, but one possibility that has been understudied is the issue of gene expression heterogeneity within patients’ tumors.

Previous studies have shown significant intratumor heterogeneity in human cervical cancer, with variations in chromosomal aberrations, microvessel density, pO2, interstitial fluid pressure and protein expression in different regions within a single patient’s tumor (11–19). Based on these many reports, our hypothesis was that there would be significant intratumoral heterogeneity in gene expression profiles in patients with
locally advanced cervical cancers. Our data, in fact, shows that the majority of genes were expressed relatively homogeneously, in that 72% of the ProbeSets had intratumoral variations of <0.5 of the total variance (Fig. 1). In an unsupervised cluster analysis, all samples except one grouped perfectly with their corresponding patient (Fig. 4), again indicating that most of the variability in fact occurred in-between different patients’ tumors.

There remained, however, significant heterogeneity among a subset of genes. An ontological analysis revealed that genes whose expression varied significantly within a tumor (high W/T), tended to fall into two broad categories; those involved with transcriptional regulation (at the level of expression as well as RNA splicing), and those involved with cellular metabolism. The high intratumor variability of these genes may reflect the changing activity of different cells within the tumor. In this context, it is worth noting that microarray data presents only a snapshot of cell activity, which obviously could change over time.

In contrast to the intratumor variability in the expression of transcription-related genes, translation-related genes, and in particular, the ribosomal genes displayed consistent expression throughout a tumor and were among the least variable genes. In addition, genes involved in cell-mediated immune response, including antigen processing and presentation, and MHC class II receptor activity genes, were also homogeneously expressed throughout the tumor. This is intriguing, as cervical cancer has a specific viral etiology, and cellular immunity likely plays a major role in cervical cancer carcinogenesis (35). In our study, genes with MHC class II receptor activity, as well as genes involved in antigen processing and presentation, were found to be one of the most homogeneously expressed genes, which could be a reflection of host factors, and therefore, less susceptible to regional differences within a tumor.

In an effort to derive some understanding of the implications of our data, we provide an estimate for the number of biopsies that would be necessary, in order to minimize the effect of intratumoral gene expression variability within a single patient’s tumor. It has been previously suggested that a reliability coefficient of at least 0.85 would be adequate to render clinical decisions based on results of diagnostic tests (33). Applying this standard to our data, this would imply that only genes with a W/T < 0.15 (n = 1,536) could be reliably estimated from a single biopsy. Consequently, genes with higher W/T (n = 18,464) would require a larger number of biopsies in order to provide a reasonable estimate of their “true” level of gene expression (see Fig. 6). For example, hypoxia-inducible factor 1α (HIF-1α) had a W/T of 0.19; hence, two biopsies from the same patients’ tumor would be necessary to obtain a more reliable estimate for this gene. In contrast, VEGF with W/T = 0.70 would require >10 biopsies. If a less stringent estimate of W/T is required (recognizing that for many useful prognostic factors, W/T may range up to 0.5), the model presented in Fig. 6 could be used to estimate the number of biopsies required, based on the initial and required W/T. However, an optimum W/T cannot be chosen on statistical reasoning alone. In the clinical world, the feasibility of taking many biopsies from one patient is a restrictive factor.

The variable proportion of tumor cells in each sample may contribute to heterogeneity in mRNA expression levels. We therefore chose to assay microarray samples with a tumor fraction of at least 50% (Supplementary Table S1; Supplementary Fig. S1). The distribution of tumor fractions is very narrow, with 31 of 33 samples having tumor fractions in the interval (0.6, 0.9). This narrowness should greatly help to reduce the influence of this confounding variable on our analysis of tumor variability, but also limits our ability to distinguish the effects of tumor heterogeneity from those of tumor cell fractions. In
Intratumor Heterogeneity in Gene Expression

Fig. 6. A model that provides an estimate for the number of biopsies necessary to obtain a reliable read-out for each gene, as a function of its W/T value. The y-axis represents the number of biopsies required; the x-axis represents the W/T for each gene. , number of biopsies that would be necessary in order to reduce heterogeneity to ≤0.15, in order to obtain a more reliable estimate for gene expression. For genes with a W/T ≤ 0.15, a single biopsy would suffice; for highly heterogeneous genes with W/T values ≥0.5, six or more biopsies would be required.

In summary, the majority of genes are expressed relatively uniformly within any single patient’s cervical tumor. However, a subset of genes involved in metabolism and transcriptional regulation can be expressed quite variably within a single cancer, indicating that for such genes, multiple samplings would be required in order to account for this heterogeneity, to fully understand their true prognostic or predictive value for cervical cancer.

Acknowledgments

University of Toronto, Department of Radiation Oncology, Radiation Research Program.

References


Gene Expression Profiling in Cervical Cancer: An Exploration of Intratumor Heterogeneity

Barbara Bachiary, Paul C. Boutros, Melania Pintilie, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/12/19/5632

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2006/10/17/12.19.5632.DC1

Cited articles
This article cites 36 articles, 11 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/12/19/5632.full.html#ref-list-1

Citing articles
This article has been cited by 14 HighWire-hosted articles. Access the articles at:
/content/12/19/5632.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.