

ASSESSING THE IMPACT OF TUMOR DEVITALIZATION TIME ON GENE EXPRESSION-BASED TISSUE OF ORIGIN TESTING

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ABSTRACT

Background: The utilization of genome-wide gene expression microarray technology in tumor stratification has proven a powerful tool to identify gene expression signatures associated with cancer prognosis as well as tissue of origin of undifferentiated metastases from unknown primary sites. Standardized protocols and guidelines for handling of tumor tissues post operatively have yet to be defined. We assessed the effect of tissue devitalization time on ovarian tumor gene expression profiling, particularly in terms of tissue of origin testing, using high-density oligonucleotide microarrays.

Materials and Methods: Residual tissue from two surgical pathology specimens, corresponding to an immature teratoma of the ovary and a serous papillary ovarian tumor, was divided into 5 samples per specimen. One of the samples, for each tumor, was immediately snap-frozen in liquid nitrogen. The remaining four samples per tumor were kept at room temperature for 15, 30, 60, and 120 minutes, at which time the tissue was snap-frozen in liquid nitrogen, and stored at -80°C until RNA extraction. Tissue of origin assessment was performed using the Pathwork® Tissue of Origin (TOO) test. For each sample, standardized expression (SE), similarity scores (SS), and physician guided conclusion (PGC) were analyzed. For the continuous variables, SE and SS, linear correlation analysis was used for each devitalization time comparison to generate Pearson's r coefficients.

Results: Very good correlations of the SE values were observed between each devitalization sample and the corresponding snap-frozen sample, with Pearson's r coefficients greater than 0.93 and 0.95 (P<0.0001) for the immature teratoma and the serous papillary ovarian tumor, respectively. Likewise, Pearson's r coefficients corresponding to SS correlations were greater than 0.88 (P<0.0001) for the immature teratoma specimen and equal to 1.00 (P<0.0001) for the serous papillary tumor. For each ovarian tumor, PGC results were identical for all devitalized and snap-frozen samples and were in agreement with the original diagnosis.

Conclusions: Our study suggests that with proper sample handling and rigorous QC procedures for RNA extraction and microarray analysis, tumor classification based on global gene expression data will not be adversely affected if devitalization times are kept within a 120-minute window.

BACKGROUND

Genome-wide gene expression microarrays have enabled the measurement of gene expression on a global scale with broad applications including the prediction of therapeutic response to chemotherapy, as well as assessment of tissue of origin of undifferentiated metastases from unknown primary sites.

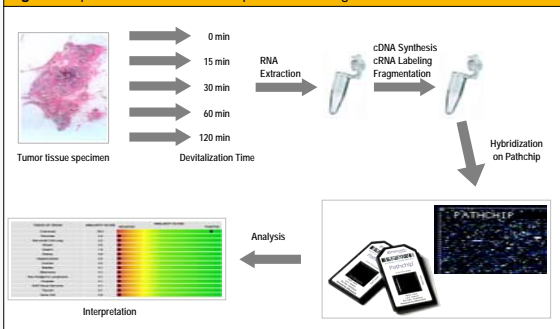
The Pathwork® Tissue of Origin Test is an in vitro diagnostic test for evaluating the tissue of origin (TOO) in poorly differentiated or undifferentiated tumors. This microarray-based gene expression test quantifies the similarity of tumor specimens to 15 tissues on the TOO Test panel by means of a proprietary machine learning algorithm trained on 2039 well-characterized tumor specimens, acquired from 14 laboratories. The tumor types included in the Pathwork TOO Test are: bladder, breast, colorectal, gastric, germ cell, hepatocellular, kidney, non-small-cell lung, non-Hodgkin's lymphoma, melanoma, ovarian, pancreatic, prostate, soft tissue sarcoma, and thyroid. The reproducibility of this test has already been evaluated on snap-frozen tissue only.¹

To ensure highly reproducible microarray results, total RNA samples, as well as cDNA and cRNA synthesis steps, need to meet rigorous quality control (QC) criteria.² It is also important to assess possible RNA degradation or changes in gene expression due to ischemia following removal of the tissue sample from the patient's blood supply, which might affect the robustness of this or any microarray-based diagnostic test.³

In the study presented here, we examined the effects of devitalization in global gene expression profiling of 2 ovarian tumors, a serous papillary (SP) and an immature teratoma (T), by freezing 5 samples per tumor specimen at different times (0, 15, 30, 60 and 120 minutes). Specifically, we aimed at assessing the impact of tissue devitalization on the Pathwork TOO Test performance.

MATERIALS AND METHODS

Figure 1. Specimen Devitalization Experimental Design and Workflow



RESULTS

Figure 2. Total RNA QA/QC

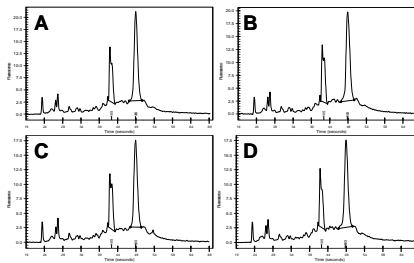


Fig. 2. Electropherograms of total RNA samples obtained from the ovarian serous papillary carcinoma (A and B), and the immature teratoma (C and D), at 0 minutes (A and C) and 120 minutes (B and D) of devitalization time.

Figure 3. Pathwork® TOO Results

Sample ID	Devitalization Time (min.)	Similarity Score (SS)	TOO	2nd Score	2nd Test	3rd Score	3rd Test	PGC
T_0	0	51.2	GC	18.1	OV	9.9	TH	GC
T_15	15	57.8	GC	13.6	OV	8.3	TH	GC
T_30	30	62.8	GC	18.1	OV	5.3	SC	GC
T_60	60	46.2	GC	36.4	OV	4.3	TH	GC
T_120	120	44.7	GC	18.0	OV	10.9	TH	GC
SP_0	0	97.7	OV	0.5	KI	0.4	TH	OV
SP_15	15	96.6	OV	1.0	KI	0.4	TH	OV
SP_30	30	98.4	OV	0.4	KI	0.2	PA	OV
SP_60	60	98.2	OV	0.4	KI	0.3	PA	OV
SP_120	120	97.5	OV	0.7	KI	0.4	LU	OV

T: immature teratoma from ovary; GC: Germ cell tumor
SP: serous papillary carcinoma of ovary; OV: Ovarian tumor
Fig. 3. Similarity Scores (SS) and Physician Guided Conclusion (PGC) obtained for all the ovarian tumor specimens with the Pathwork TOO Test.

Figure 4. Similarity Scores (SS)

Immature teratoma from ovary					
Time	0	15	30	60	120
TOO SS	51.2	57.8	62.8	46.2	44.7
2nd Score	18.1	13.6	18.1	36.4	18
3rd Score	9.9	8.3	5.3	4.3	10.9
Pearson's r =		0.996	1.000	0.806	1.000

Serous papillary carcinoma of ovary					
Time	0	15	30	60	120
TOO SS	97.7	96.6	98.4	98.2	97.5
2nd Score	0.5	1	0.4	0.4	0.7
3rd Score	0.4	0.4	0.2	0.3	0.4
Pearson's r =		1.000	1.000	1.000	1.000

Fig. 4. Pearson correlation between each devitalization time point against the snap-frozen sample, for the top 3 Similarity Scores (SS) obtained with the Pathwork TOO Test for **A)** Immature teratoma from the ovary and **B)** Serous papillary carcinoma of the ovary.

Figure 5. Similarity Scores (SS) and Standardized Expression (SE)

		Pearson's r									
SS	SE	T_0	T_15	T_30	T_60	T_120	SP_0	SP_15	SP_30	SP_60	SP_120
T_0		1.000	0.958	0.955	0.951	0.956	0.654	0.680	0.675	0.671	0.674
T_15		0.992	1.000	0.961	0.952	0.965	0.659	0.684	0.679	0.674	0.677
T_30		0.991	0.995	1.000	0.962	0.944	0.679	0.702	0.701	0.691	0.693
T_60		0.927	0.883	0.912	1.000	0.937	0.688	0.710	0.711	0.706	0.704
T_120		0.996	0.983	0.980	0.932	1.000	0.643	0.665	0.665	0.658	0.659
SP_0		0.240	0.131	0.195	0.578	0.271	1.000	0.957	0.961	0.967	0.963
SP_15		0.239	0.130	0.194	0.577	0.270	1.000	0.960	0.964	0.966	0.964
SP_30		0.239	0.130	0.194	0.577	0.270	1.000	1.000	0.976	0.965	0.965
SP_60		0.239	0.130	0.194	0.577	0.270	1.000	1.000	1.000	0.979	0.979
SP_120		0.238	0.130	0.194	0.577	0.270	1.000	1.000	1.000	1.000	1.000

T: immature teratoma from ovary
SP: serous papillary carcinoma of ovary
Fig. 5. Pearson correlation of the 15 TOO Similarity Scores (below identity line) and the 1668 Standardized Expression (SE) values (above identity line) between each ovarian tumor sample. The grey boxes indicate the identity line and the red numbers correspond to correlation coefficients obtained from sample comparisons within the same ovarian tumor specimen.

CONCLUSIONS

The microarray-based Pathwork® TOO Test is intended to be used with total RNA isolated from snap-frozen tissue samples, thus there is a need to assess possible RNA degradation or changes in gene expression due to ischemia following removal of the tissue sample from the patient's blood supply, which might affect the robustness of this or any microarray-based diagnostic test.

In this study, we assessed the impact of tissue devitalization on the Pathwork TOO Test performance performed on 2 ovarian tumors, a serous papillary and an immature teratoma, by freezing 5 samples per tumor specimen at different times (0, 15, 30, 60 and 120 minutes).

The results of this study showed that no visible total RNA degradation was observed even after 120 minutes of devitalization on both ovarian tumor specimens. In addition, when compared to the snap-frozen sample (Time 0), all the devitalized samples showed a very good correlation (Pearson's r > 0.8) on the top 3 similarity scores (SS) obtained, and identical physician guide conclusion (PGC) tissue type results, all of them in agreement with the original diagnosis.

Moreover, very good correlations of the standardized expression (SE) values for the 1668 probe sets on the PathChip® array were observed for all the samples within the same specimen, regardless of the freezing time, with Pearson's r coefficients greater than 0.93 and 0.95 (P < 0.0001) for the immature teratoma and the serous papillary ovarian tumor, respectively. Likewise, Pearson's r coefficients for all the samples based on the 15 SS were greater than 0.88 (P < 0.0001) for the immature teratoma specimen and equal to 1.00 (P < 0.0001) for the serous papillary tumor.

Altogether, these results strongly suggest that with proper sample handling and rigorous QC procedures for RNA extraction and microarray analysis, tumor classification based on global gene expression data will not be adversely affected if devitalization times are kept within a 120-minute window.

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