Identification of a Cardiac Allograft Rejection Marker Using Microarray Gene Expression Analysis in Lymphocytes From Heart Transplant Patients

To the Editor,

The diagnosis of graft rejection is one of the fundamental aspects of caring for patients with heart transplantation. Endomyocardial biopsy (EMB) has important limitations, as it is an invasive procedure, with variable interpretation and limited sensitivity.[1-3] Thus, it would be of interest to have a noninvasive diagnostic method that facilitates follow-up of patients with heart transplantation.

The gene expression patterns of peripheral blood lymphocytes may represent a highly sensitive, highly specific tool that would identify patients who present rejection and avoid the inconvenience of EMB. We present the preliminary results of a study performed to determine a set of candidate genes for use in a noninvasive molecular expression test with peripheral blood to detect cardiac graft rejection.

The experiments to analyze gene expression were conducted with 6 peripheral blood samples taken from heart transplant recipients on the day of EMB. Rejection was diagnosed according to the International Society of Heart and Lung Transplantation (ISHLT) classification.[4] Three of the samples corresponded to patients with rejection (ISHLT Grade ≥2 ISHLT rejection) and three to patients without rejection (ISHLT Grade 0 rejection). The blood samples were collected in tubes containing a solution for RNA stabilization (PAXgene Blood RNA Tubes, Qiagen) and stored at –80ºC until the time of analysis.

Genetic tests were performed with Affymetrix microarrays (GeneChip Human Genome U133 Bonus 2.0 Array). Differences in the expression of 262 genes were found when using the GeneSpring comparison algorithm. Two-dimensional hierarchical cluster allows verification of the presence of differential expression patterns consisting of samples with rejection compared to the control samples. Of these 262 genes, Table lists the candidate genes.

Five metabolic pathways with at least 2 genes of modified expression level were found. These metabolic pathways were:

TABLE 1. **List of 23 Genes in Which a Significant Modification in Gene Expression Was Found***

Systematic	P-value	Fold Change	Gene title	UniGene ID
202483_s_at	.0362	0.72	RAN binding protein 1	24763
222435_s_at	.0329	0.38	Ubiquitin-conjugating enzyme E2, J1 (UBC6 homolog, yeast)	163776
204115_at	.0329	1.83	Guanine nucleotide binding protein (G protein), gamma 11	83381
204081_at	.0327	2.27	Neurogranin (protein kinase C substrate, RC3)	524116
213095_x_at	.0316	0.56	Allograft inflammatory factor 1	76364
200087_s_at	.0315	0.43	Coated vesicle membrane protein /// coated vesicle membrane protein	75914
209201_x_at	.0312	0.62	Chemokine (C-X-C motif) receptor 4	421986
217877_s_at	.0308	0.62	Hypothetical protein SP192	238432
200614_at	.0303	0.56	Clathrin, heavy polypeptide (Hc)	491351
224644_at	.0302	0.47	Homo sapiens, clone IMAGE:5278517, mRNA	517821
201453_x_at	.0264	0.63	Ras homolog enriched in brain	283521
228959_at	.026	0.64	CDNA clone IMAGE:5262734, partial cds	296031
207305_s_at	.0259	0.55	KIAA1012	202001
212429_s_at	.0256	0.53	General transcription factor IIIC, polypeptide 2, beta 110kDa	75782
1557905_s_at	.0254	0.58	CD44 antigen (homing function and Indian blood group system)	502328
200729_s_at	.0232	0.37	ARP2 actin-related protein 2 homolog (yeast)	393201
200009_at	.023	0.45	GDP dissociation inhibitor 2 /// GDP dissociation inhibitor 2	299055
225225_at	.023	0.46	Homo sapiens, clone IMAGE:5274897, mRNA	351680
200607_s_at	.0229	0.58	RAD21 homolog (S. pombe)	81848
227621_at	.0226	0.36	Wilms tumor 1 associated protein	446091
202603_at	.0202	0.35	A disintegrin and metalloproteinase domain 10	172028
217825_s_at	.0123	0.56	Ubiquitin-conjugating enzyme E2, J1 (UBC6 homolog, yeast)	163776
239205_s_at	.00254	1.69	Complement component (3b/4b) receptor 1	334019

*The first column, Systematic, refers to the probe identification number in the U133plus Affymetrix chip. *P*-value is the significance value of the expression change observed. The fold change indicates the degree of expression change (1 represents no change, <1 indicates repression, >1 overexpression of gene). Gene title is the name of the gene. UniGene ID is the identifier of this gene in the GenBank database.

apoptosis (4 genes), MAP-kinase pathway (5 genes), B cell receptor signaling pathway (2 genes), hematopoietic cell lineage (2 genes), signaling pathway, and T-cell receptor signaling pathway (2 genes).

The results are consistent with the hypothesis of immune system stimulation implicated in the rejection of transplanted organs. Horwitz et al[5] used a similar method, finding that *CFLAR*, a gene implicated in the apoptotic pathway, was altered in patients with transplant rejection. Similar results were obtained by Deng et al,[6] who found alterations in the expression of genes related to the MAP-kinase pathway, apoptosis, and T-cell receptors.

In conclusion, this exploratory study in heart transplant patients found differences in gene expression between patients with and without rejection. Of the five pathways showing genes with altered expression, the apoptotic and MAP-kinase pathways appeared to have the highest number of affected genes. The low number of study samples limits the interpretation of the findings. Nevertheless, we consider that the results of this preliminary work justify the expectations placed in this promising strategy, which may contribute to the development of a molecular test to monitor immunosuppressive therapy in patients who have undergone transplantation.

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REFERENCES

1. Hertz MI, Boucek MM, Deng MC, Edwards LB, Keck BM, Kirklin JK, et al. Scientific Registry of the International Society for Heart and Lung Transplantation: introduction to the 2005 annual reports. J Heart Lung Transplant. 2005;24:939-44.
2. Pophal SG, Sigfusson G, Booth KL, Bacanu SA, Webber SA, Ettedgui JA, et al. Complications of endomyocardial biopsy in children. J Am Coll Cardiol. 1999;34:2105-10.
3. Marboe CC, Billingham M, Eisen H, Deng MC, Baron H, Mehra M, et al. Nodular endocardial infiltrates (Quilty lesions) cause significant variability in diagnosis of ISHLT Grade 2 and 3A rejection in cardiac allograft recipients. J Heart Lung Transplant. 2005;24 Suppl 7:219-26.
4. Stewart S, Winters GL, Fishbein MC, Tazelaar HD, Kobashigawa J, Abrams J, et al. Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection. J Heart Lung Transplant. 2005;24:1710-20.
5. Horwitz PA, Tsai EJ, Putt ME, Gilmore JM, Lepore JJ, Parmacek MS, et al. Detection of cardiac allograft rejection and response to immunosuppressive therapy with peripheral blood gene expression. Circulation. 2004;110:3815-21.
6. Deng MC, Eisen HJ, Mehra MR, Billingham M, Marboe CC, Berry G, et al. Noninvasive discrimination of rejection in cardiac allograft recipients using gene expression profiling. Am J Transplant. 2006;6:150-60.