Gene Expression Profiling in Multiple Myeloma—Reporting of Entities, Risk, and Targets in Clinical Routine

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Abstract

Purpose: Multiple myeloma is an incurable malignant plasma cell disease characterized by survival ranging from several months to more than 15 years. Assessment of risk and underlying molecular heterogeneity can be excellently done by gene expression profiling (GEP), but its way into clinical routine is hampered by the lack of an appropriate reporting tool and the integration with other prognostic factors into a single “meta” risk stratification.

Experimental Design: The GEP-report (GEP-R) was built as an open-source software developed in R for gene expression reporting in clinical practice using Affymetrix microarrays. GEP-R processes new samples by applying a documentation-by-value strategy to the raw data to be able to assign thresholds and grouping algorithms defined on a reference cohort of 262 patients with multiple myeloma. Furthermore, we integrated expression-based and conventional prognostic factors within one risk stratification (HM-metascore).

Results: The GEP-R comprises (i) quality control, (ii) sample identity control, (iii) biologic classification, (iv) risk stratification, and (v) assessment of target genes. The resulting HM-metascore is defined as the sum over the weighted factors gene expression–based risk-assessment (UAMS-, IFM-score), proliferation, International Staging System (ISS) stage, t(4;14), and expression of prognostic target genes (AURKA, IGF1R) for which clinical grade inhibitors exist. The HM-score delineates three significantly different groups of 13.1%, 72.1%, and 14.7% of patients with a 6-year survival rate of 89.3%, 60.6%, and 18.6%, respectively.

Conclusion: GEP reporting allows prospective assessment of risk and target gene expression and integration of current prognostic factors in clinical routine, being customizable about novel parameters or other cancer entities. Clin Cancer Res; 17(23); 7240–7. ©2011 AACR.
Translational Relevance

Multiple myeloma is an example for a cancer entity characterized by a pronounced molecular heterogeneity, transmitting into survival times ranging from months to more than 15 years. Gene expression profiling is an ideal tool to assess this heterogeneity in terms of gene expression–based biologic classifications and prognosis and allows assessment of target genes. However, it is almost never used prospectively in clinical routine, mainly due to the lack of an academic reporting tool giving quality controlled, validated, and clinically digestible information.

We present here a gene expression–based report. The gene expression–based report is a customizable opensource software that can be adapted to novel parameters or other cancer entities. It includes molecular classification, risk stratification, and assessment of target gene expression. Furthermore, the HM-metascore combines validated conventional and expression-based risk factors into one superior metascore, thus helping to overcome the confusing and increasing multitude of prognostic factors.

Materials and Methods

Software design

The GEP-R software is developed within the open-source environment R (21) and Bioconductor (22) using a graphical user interface (GUI; see Supplementary Fig. S1) based on the gWidgets package (23). It processes a single Affymetrix U133 Plus 2.0 .CEL file using a “documentation by value (docval)” strategy (24) modified for GC-RMA preprocessing (available at http://code.google.com/p/gep-r). This allows using preprocessing information of our reference cohort of 262 quality-controlled myeloma samples (E-MTAB-372) and thus generated thresholds to be applied to a subsequent microarray, a prerequisite for prospective use of a gene expression report based on GC-RMA preprocessed data. A detailed description of the GUI and how to use it is provided in the Supplementary Materials and Methods.

Quality control

Quality control (QC) implemented is based on the Bioconductor packages affyQCReport (25), affyPLM (26–28), simpleaffy, and yaqcaffy (29) adapted for GC-RMA preprocessing and PANP-based (30) assessment of presence/absence of gene expression. To limit computation time, 6 samples from the reference cohort have been chosen randomly as QC reference.

Sample validation

Using prediction analysis for microarrays [PAM; (31)], predictors for sex, the IgL- (lambda and kappa), and IgH-type (IgA, IgG, IgD) have been implemented using one part of the reference cohort as training set and the other one as test set. For further validation, the UAMS total therapy 2 (TT2) cohort (GSE24080) and the Multiple Myeloma Research Consortium data (MMRC; www.broadinstitute.org/mmgp/home) were used.

Risk stratification

Two gene expression–based risk scores and a risk delineating GPI are implemented: (i) The IFM 15-gene model (15) developed on one-channel DNA Unité Mixte de Génomique du Cancer (UMGC) microarrays transferred to respective genes represented on Affymetrix U133 Plus 2.0 arrays. Cutoff points for low/high risk have been generated on the reference cohort defining the 25% of patients with the highest risk score as high risk, showing comparable results with the original publication (15). (ii) The IFM 15-gene risk score was calculated as published on MAS5 preprocessed data using published cutoff points (14). (iii) The GPI (16) with cutoff values for low/medium/high risk was regenerated on the reference cohort. The attribution of the TT2 cohort to the respective risk stratification can be found in Supplementary Table S1.

Assessment of gene expression

Expression height and presence/absence of expression by PANP (30) is assessed for (i) potential “target genes” for whose products clinical grade inhibitors exist (currently AURKA, FGFR3, and IGF1R), (ii) potential vaccination targets, and (iii) genes frequently aberrantly (i.e., not expressed in normal plasma cells) or differentially expressed in myeloma (i.e., expression value greater/lower than the...
Gene expression–based classification of myeloma

The following 3 gene expression–based classifications of multiple myeloma are assessed: (i) The TC classification by Bergsagel and colleagues (10, 11) derived on Hu95Av2 DNA microarrays (Affymetrix) using MAS5 preproposition was implemented on U133 2.0 (Affymetrix) DNA microarrays as described by Chng and colleagues (32). (ii) The molecular classification of multiple myeloma (9): We first carried out an unsupervised clustering of the reference cohort based on the 700 genes published (being de facto 687 genes). The resulting 7 clusters could be attributed to the 7 groups of the molecular classification. A “myeloid group” could not be delineated (see Results). A PAM-based predictor using these 687 genes was calculated on 162 patients of the reference cohort and validated on 100 patients, respectively. (iii) The EC classification (12) was reimplemented on the reference cohort and assessed by a PAM predictor consisting of 188 genes. The attribution of the TT2 patients to the respective gene expression–based classifications can be found in Supplementary Table S1.

Chromosomal aberrations—presence of t(4;14)

The presence of a translocation t(4;14) has been assessed by interphase FISH as previously published (16) and a PAM-based predictor using parts of the reference cohort as training set and the other part as test set has been generated (36 genes).

Calculation of the HM-metascore

The calculation of the HM-metascore is described in the Results section (see later).

Results

GEP-R

After input of patient information (e.g., sex, IgH/L-type), the GEP-R software carries out MAS5 and GC-RMA preprocessing as detailed above and subsequently applies the following: (i) quality control of gene expression data, (ii) sample validation, (iii) gene expression–based classification, (iv) risk stratification, and (v) target assessment. The physician preparing the report comments respective findings within the report in an editable text field. A PDF file containing the final report is generated by the software using the Sweave package (33). The GEP-R consists of the following 2 parts: (i) the report given to the treating physician (Fig. 1) and (ii) an appendix containing detailed quality control and validation information, as well as details of the assessment of gene expression (Supplementary Fig. S2). Analyzing a .CEL file with GEP-R takes about 20 minutes on a standard computer (2+ GB RAM required). The GEP-R runs on Linux, MacOS, and Windows systems. Optionally, results and gene expression data can be written to a PostgreSQL database using PostgreSQL through pgUUtils (34) and maDB (35) packages, provided a running PostgreSQL database. For metascore generation (HM-score, see below), the patient’s International Staging System (ISS) stage needs to be entered.

Description of the GUI

The GEP-R GUI is divided into 2 parts (Supplementary Fig. S1). Left part: Within the left part of the GUI, the user (i) loads an Affymetrix U133 Plus 2.0 raw file into the software, starts the analysis, can save or load previous analyses, and creates the GEP-R as a PDF document. The user enters (ii) patient- and (iii) sample-specific data (e.g., patient name, IgH-/IgL-type) necessary for identification and validation. Afterward, the .CEL file is analyzed by the GUI calling an R-script carrying out MAS5 and GC-RMA preprocessing using preprocessed information of the reference cohort. The user here (iv) comments the parameters within the report and (v) includes a concluding statement. Right part: here, the results of the analysis are presented in 3 tabs for (i) parameters analyzed (e.g., risk scores), (ii) quality control parameters and plots, and (iii) identity control parameters that appear if entered patient data do not match predicted values (e.g., IgH-type), or a data field misses an entry (Supplementary Figs. S2 and S3). A detailed description of the GUI and how to use it is included in the Supplementary Materials and Methods.

Exemplary report

An example of a GEP-R (final PDF-based patient report given to clinicians and patients) is depicted in Fig. 1, the full report including the appendix in Supplementary Fig. S2.

Quality control

QC metrics assessed are average background, percentage of present calls as detected by the PANP algorithm, 3', mid and 5' expression of β-actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Six plots allow the assessment of reproducibility, QC statistics, spike-in performance, normalized unscaled standard error (NUSE), relative log expression (RLE), pseudo images, for example, allowing the detection of artifacts, and RNA degradation (Supplementary Fig. S3). Supplementary Table S2 depicts samples from the initial cohort of 280 patients failing these criteria. In all, 18 samples (6.4%) failed in at least one criterion and were excluded. One criterion was failed by 10 (3.5%), 3 criteria by additional 4 (1.4%), and 3 or more by 4 (1.4%) further samples, respectively.

Validation

The GEP-R software compares predicted sex, IgH-, and IgL-type with user-entered patient information and gives a warning in case of inconsistency. For prediction errors, see Supplementary Table S3. Predictors were validated on the UAMS TT2 cohort and the MMRC data set (Supplementary Table S3).
Risk stratification

Using the GEP-R–based assessment, the gene expression–based risk scores of the UAMS and IFM significantly delineate a high-risk population of 29.2% and 22.9% of patients with myeloma, respectively. The same holds true for the GPI significantly delineating 6.3%, 47.9%, and 45.8% of patients as high, intermediate, and low risk (Supplementary Fig. S4). The risk stratification according to the UAMS 70-gene score for the UAMS patients is available as Supplementary Table S1.

Assessment of target gene expression

On our reference cohort, the assessed target genes AURKA, IGF1R, and FGFR3 are expressed in 29.3%, 28.2%, and 13.4% of myeloma samples, respectively. Expression of each gene is significantly associated with adverse survival on our cohort (Supplementary Fig. S4). For the reporting, see Supplementary Figs. S1 and S2.

Gene expression–based classification of myeloma

The TC classification is implemented as suggested by the authors (ref. 32; see above). Grouping is validated by published data of the TT2 data set (Supplementary Table S1). The EC classification implemented as stated above can be predicted with an overall error rate of 3% (Supplementary Table S3B). In contrast, grouping according to the “molecular classification” proposes a problem as there is no clear delineation algorithm of the so-called “myeloid group” published. Nevertheless, the molecular classification (7 groups, without the “myeloid group”) can be predicted with an overall error rate of 11%. For details, see Supplementary Table S3B. The overlap of our prediction with the grouping according to Zhan and colleagues (9) is visualized in Supplementary Table S1.

Chromosomal aberrations—presence of t(4;14)

The presence of a translocation t(4;14) can be assessed using a PAM-based predictor without error (Supplementary Table S3C). Its expression is significantly associated with adverse prognosis (Supplementary Fig. S4).

Building a metascore including current prognostic information

To integrate depicted expression-based and clinical prognostic factors within one prognostic (meta-) score...
(HM-score), a weight of 0, 0.5, or 1 is given for each of the named prognostic factors. The HM-score is calculated as the sum over these weights for gene expression–based assessment of risk (0, 0.5, 1 for none, one, or both of the UAMS- and IFM-scores depicting high risk, respectively), proliferation (0, 0.5, 1 for GIPI low, median, or high, respectively), ISS (0, 0.5, 1 for ISS stage 1, 2, and 3, respectively), t(4;14) (0 for absence, 1 for presence of the aberration), and expression of prognostically relevant target genes (0 or 1 if none or at least one of the genes AURKA and IGF1R). A, significant delineation of event-free survival (EFS) within our (HM cohort) and the TT2 cohort. (HM cohort, median, medium, and high risk; TT2 cohort, medium, low, and high risk; P < 0.001, low vs. medium, P < 0.001, medium vs. high, P < 0.001; TT2 cohort, medium, low, and high risk; P < 0.001, respectively). B, significant delineation of overall survival (OS; HM cohort, low vs. medium, medium vs. high, P < 0.001; TT2 cohort, low vs. medium, medium vs. high, P = 0.006, medium vs. high, and P < 0.001, respectively).

**Discussion**

In this article, we introduce a reporting tool (GEP-R) allowing automated interpretation of Affymetrix U133 Plus 2.0 gene expression profiles. It enables the prospective use of GEP within clinical routine in terms of molecular classification, risk stratification, and assessment of target genes. This represents the critical first step in a process that will...
eventually allow for reproducible approaches and a standardization of the analyses across different centers as well as for personalized treatment.

In 2009, the International Myeloma Working Group stated: "Our group also recognizes that the greatest prognostic ability for multiple myeloma resides in the comprehensive analysis of GEP. At a minimum, all clinical trials should consider incorporation of GEP into the correlative science studies to identify subgroups of high-risk disease. We also propose that methodology to include GEP into the clinical testing is urgently needed and methods for implementation should be identified" (16). In the following, we discuss use, interest in, and clinical applicability of GEP and the GEP-R, respectively.

Three gene expression–based classifications delineate molecular groups in myeloma: The "molecular classification" based on differential gene expression in which 3 of 7 groups ("proliferation," MAF expression, MMSET overexpression) show different survival (9); the TC classification based on translocations and D-type cyclin without prognostic relevance (10, 11) and EC classification based on chromosomal aberrations and resulting changes in gene expression with only 1 of 4 groups (t(4;14) and FGFR3 expression] showing adverse prognosis (12, 13). Biologic classifications likely remain relatively stable in contrast to prognostic factors prone to change with different treatment schedules (see below).

The translocation (t;4;14) represents a specific disease entity and is an independent risk factor despite conventional or high-dose treatment (36–41). Treatment with bortezomib or lenalidomide containing regimen seems to be of "high risk." As implemented in the GEP-R, 2 additional or high-dose treatment (36–41). Treatment with bortezomib or lenalidomide containing regimen seems to be of "high risk." As implemented in the GEP-R, 2 additional

discuss use, interest in, and clinical applicability of GEP and the GEP-R, respectively.

In conclusion, gene expression profiling using GEP-R allows prospective reporting of molecular classifications, risk, and therapeutic targets to clinicians in clinical routine in a digestible manner and thus represents a major step in translational oncology. It will foster carrying out clinical trials using risk-adapted and personalized treatment strategies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

T. Meißner carried out programming and creation of the GEP-R and participated in writing of the manuscript; A. Seekinger participated in designing the research and writing of the manuscript; T. Rème participated...
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in the creation of the GEP-R, T. Hielscher participated in statistical analysis and the modification of the docval-package; T. Möhler, K. Neben, B. Klein, and H. Goldschmidt participated in the analyzing of the data and in the writing of the manuscript; and D. Hose designed research, wrote the manuscript, and participated in creating the GEP-R.

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References


10. Hose D, Rossi J-Fo, Iltring C, Deves O, Fizier M, Benner A, et al. Molecular classification of multiple myeloma (MM) based on gene expression profiling (GEP) and fluorescence in situ hybridisation ISH. In: The role of GPR1 as a major growth factor for myeloma cells and the modification of the docval-package; T. M


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