TeratoScore: Assessing the Differentiation Potential of Human Pluripotent Stem Cells by Quantitative Expression Analysis of Teratomas

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Supplementary Figure 1. Representative Histological Sections of Teratomas Showing Structures from All Three Germ Layers. The teratomas reported in this paper were heterogeneously comprised, with structures originating from endoderm, mesoderm and ectoderm lineages (marked with arrows). 4-µm sections of teratomas stained with hematoxylin and eosin are shown. The teratomas were initiated from CSES32 (top right), CSES21 (top left), CSES26 (bottom left) and CSES25 (bottom right) cells. Abbreviations: END: Endoderm, MES: Mesoderm, ECT: Ectoderm.
Supplementary Figure 2. CellNet Online Resource Output for hESC, Teratoma and Tumor data. Expression data sets from four human embryonic stem cell lines (ESCs), 14 teratomas and seven different somatic tumors were uploaded to the online CellNet resource (Cahan et al., 2014). While ESCs show a clear resemblance to the ESC categories, all teratomas do not show a distinct similarity to any of the cell types measured, as shown in both a heat map analysis of the individual data sets (A) and histograms of gene regulatory networks found in each teratoma-group representing different cell types (B). Somatic tumors do show a distinct classification according to their initiating tissue (A). Abbreviations: ESC: embryonic stem cells; BSC: basal cell carcinoma, ALL: acute lymphoblastic leukemia, HCC: hepatocellular carcinoma, CC: choriocarcinoma, HSC: Hematopoietic Stem Cells, S. Muscle: Skeletal Muscle.
Supplementary Figure 3. TeratoScore Grades of Different Normal Tissues. Average tissue gene expression was calculated in respect of its expression values in the original tissue. Lineage expression was calculated as the mean expression of all genes representing tissues in that lineage. Error bars represent standard error. Each tissue shows a correct specific tissue and lineage expression. All tissues produced a low TeratoScore value, reflecting the lack of pluripotent origin.
Supplementary Table 1. Teratoma Expression Profiles’ GEO Sample IDs. Cell lines used to initiate the teratomas used in this study are listed, as well as their karyotype classification and Gene Expression Omnibus (GEO) sample numbers.

| Cell line | Karyotype | Geo Series ID | GEO Sample ID | Reference |
|-----------|-----------|---------------|----------------|-----------|
| CSES7     | 46, XX    | GSE51455      | GSM1246012     | *         |
| CSES12    | 46, XX    | GSE64669      | GSM1576685     | This Study|
| CSES15    | 46, XX    | GSE51455      | GSM1246013     | *         |
| CSES26    | 46, XX    | GSE51455      | GSM1246015     | *         |
| CSES25    | 46, XX    | GSE51455      | GSM1246014     | *         |
| CSES22 (p16) | 46, XX + 12 | GSE64669 | GSM1576686 | This Study |
| CSES22 (p35) | 46, XX + 12 | GSE51455 | GSM1246017 | *         |
| CSES45 (p20) | 46, XX + 12 | GSE51455 | GSM1246018 | *         |
| CSES45 (p30) | 46, XX + 12 | GSE64669 | GSM1576687 | This Study |
| HiPS18    | 46, XX + 12 | GSE51455 | GSM1246019 | *         |
| CSES13    | 46, XX + 21 | GSE64669 | GSM1576688 | This Study |
| CSES20    | 46, XX + 21 | GSE64669 | GSM1576689 | This Study |
| CSES21    | 46, XX + 21 | GSE64669 | GSM1576690 | This Study |
| CSES32    | 46, XX + 21 | GSE64669 | GSM1576691 | This Study |

* Ben-David et. al, 2014
**Supplementary Table 2. Expression Profiles of Tissues and Tumors Used in this Study.**
Expression profiles of tissues representing the three germ layers, the placenta and embryonic stem cells used to develop the TeratoScore are listed with their Gene Expression Omnibus (GEO) sample and series IDs. Tumors expression profiles used to compare TeratoScores are also listed with their Gene Expression Omnibus (GEO) sample and series IDs.

**Supplementary Table 3. Genes Enriched for Tissues from Three Germ Layers and Placenta Used to Calculate TeratoScore.** 100 genes were selected to represent tissues from all three germ layers and extraembryonic tissue in order to calculate tumors’ TeratoScore. Genes enriched for each tissue were selected computationally and verified as being tissue-specific using Amazonia! online database (Le Carrou et al., 2010). Tissues with similar gene-expression were merged into a single, larger category. An additional set of ten genes representing pluripotent markers was selected. These genes are not taken into account when calculating TeratoScore. Listed are gene symbols, approved names and chromosomal locations.

**Supplementary Table 4. Tissue-Specific Genes Up- and Downregulated in Teratomas Initiated from Aneuploid Pluripotent Cells.** Tissue-specific genes differentially expressed in chromosomally aberrant teratomas compared to teratomas initiated from diploid cells. Out of the top 200 expressed genes in each tissue, a gene was considered differentially expressed when changed over 2-fold (either upregulated or downregulated) compared to control.
Supplemental Experimental Procedures

Cell Culture. Human embryonic stem cell lines CSES7, CSES 12, CSES13, CSES15, CSES20, CSES21, CSES22, CSES25, CSES26, CSES32 and CSES45 (Biancotti et al., 2010; Narwani et al., 2010) and iPSC line hiPS18 (Chin et al., 2009) were cultured using standard conditions as previously described (Mayshar et al., 2010). In brief, cells were grown on mouse embryonic fibroblast feeder (MEF) layer in KnockOut Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 15% KnockOut SR (Invitrogen), 1 mM glutamine, 0.1 mM b-mercaptoethanol (Sigma-Aldrich), 1% nonessential amino acids stock (Biological Industries), penicillin (50 U/ml), streptomycin (50 µg/ml) and FGF2 (4 ng/ml, Invitrogen). Cells were passaged using 0.25% trypsin solution (Biological Industries).

Karyotyping. A confluent, 10-cm plate of hESCs was used to analyze each cell line. Cells culture media were supplemented with KaryoMAX colcemid (100 ng/ml, Biological Industries) for 30 min at 37°C (with 5% CO2). Cultures were then trypsinized, treated with hypotonic solution and fixed using methanol:acetic acid solution (3:1). Metaphases were spread on glass slides and chromosomes were stained using standard G-bandig technique. Chromosomal classification was made using GenASIs software (Applied Spectral Imaging) according to the ISCN (International System for Human Cytogenetic Nomenclature).

Tumor Formation and Analysis. Undifferentiated hPSC colonies cultured on MEFs were trypsinized into single cells. ~3x106 cells were re-suspended in 50–100 µl of phosphate buffered saline (PBS) and injected under the kidney capsule of 6- to 8-week-old NOD-SCID mice. Three to four weeks post-injection, mice were sacrificed and resulting teratomas were extracted and dissected. Tumors were dissected into smaller pieces from different areas. Half of the tumor pieces were mixed together and taken for RNA extraction while the rest were saved for H&E staining, carried out as previously described (Kopper et al., 2010). The RNA portion was immediately homogenized in RLT buffer (RNeasy Mini kit, Qiagen) using a Polytron PT 1200 E system (Kinematica).
**Microarray and Data Analysis.** Total RNA was extracted from hPSC-derived teratomas using RNeasy mini kit (Qiagen), according to the manufacture instruction. RNA was subjected to Human Genome U133 Plus 2.0 microarray platform (Affymetrix); washing and scanning were performed according to the manufacturer’s protocol. Original microarray data are accessible at the NCBI Gene Expression Omnibus database under the accession numbers GSM1576685 and GSE51455 (Table S1). Microarray data of the various body tissues, hPSC and previously published teratomas and tumors were downloaded from the Gene Expression Omnibus database (Table S2). Arrays were normalized using RMA algorithm in the Affymetrix Expression Console. Probe sets with expression values lower than 20 were raised to this level. The karyotype of the analyzed in-house cell lines was validated by virtual karyotyping (Ben-David et al., 2013).

Following averaging of samples from the same tissue, an expression ratio was calculated between each tissue and the average of all other body tissues, indicating tissue-specificity. Scorecard genes for each tissue were chosen by having a high tissue specificity (over 3 folds) and by being expressed in teratoma but not in hPSCs. Tissue-specific gene expression was further validated using Amazonia!, with a requirement for distinct tissue expression (an order of magnitude over all or most other tissues) (Le Carrour et al., 2010). Gene lists for central and peripheral nervous system, small intestine and colon, fetal brain and brain and fetal liver and liver were each merged together, as their specific-gene expression was considerably similar. Average expression for each lineage was calculated as the mean expression of all genes representing that lineage. TeratoScore grades were calculated as the multiplication of these means and dividing this product by 103. One-tail t-tests between tissue expressions were done using alpha 0.05. Top-200 tissue enriched genes were chosen as the top unique genes in the tissue/body ratio mentioned above. Genes were considered differentially expressed when changed over 2-fold compared to WT. To determine significance a chi-square independence test was performed followed by Bonferroni correction for multiple testing. Statistical calculations and figures were made in R.
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