RT-PCR

RNA isolation and RT-PCR were performed as previously described (Nori et al., 2011; Okada et al., 2008).

Transcriptome Analysis

Total RNA from each sample was purified as previously described (Okada et al., 2008). mRNA libraries were prepared according to the TruSeq RNA sample prep kit protocol and sequenced using a Genome Analyzer IIx (Illumina). Mouse and human mRNA sequences were separated using Xenome software (Conway et al., 2012), and separated mRNA-seq data were mapped to the corresponding genomic DNA sequences (human [hg19] and mouse [mm9]) using TopHat software (Trapnell et al., 2009). The mapped sequences were normalized by trimmed mean of M values (TMM) and analyzed using Avadis NGS software (Agilent Technologies). All software used default parameters. For principal component analysis (PCA) and clustering analysis, the normalized data were narrowed down to 13,693 genes using a cutoff value for expression levels (reads per kilobase of exon per million mapped reads [RPKM] > 100). A Venn diagram was constructed to visualize the 1,715 genes that were upregulated in the 253G1-NS/TP-103d group and the 1,358 genes that were upregulated in the 201B7-NS/TP-103d group (RPKM > 100, fold change > 5.0 versus each NS group). GO analysis was performed using gene lists from the overlapping area, as well as from each separate area, in the Venn diagram. For GO analysis, p values were calculated using Fisher's exact test. Subsequently corrected p values were applied for multiple testing corrections using the Benjamini-Yekutieli method with a cutoff at $p = 0.05$. Pathway analysis was performed via IPA (Ingenuity Systems) using genes that were up- or downregulated in the 253G1-NS/TP-103d group versus the 201B7-NS/TP-103d group, as well as in 253G1-NS versus 201B7-NS (RPKM > 100, fold-change > 3.0). The genes were overlaid on the Ingenuity Knowledge Database and networks were algorithmically generated based on their connectivity. The p values were determined using Fisher's exact test and $p = 0.05$ was interpreted as indicating statistical significance.

Statistical Analyses

An unpaired, two-tailed Student's t test was used to assess the 253G1-NS differentiation efficacy. One-way ANOVA followed by the Tukey-Kramer test for multiple comparisons was used for the Ki-67, Rotarod, and DigiGait analyses. Repeated-measures, two-way ANOVA followed by the Tukey-Kramer test was used for the BMS analysis. The Kruskal-Wallis non-parametric test and Scheffe's test were used to analyze tumor diameter and the percentage of HNu^+ grafted cells that were $Nestin^+$, $Ki\text{-}67^+$, or $OCT4^+$. Statistical significance was determined as $^*p < 0.05$, $^{**}p < 0.01$.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, two figures, and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.stemcr.2015.01.006.

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