CORRIGENDUM

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Corrigendum: TCF3 alternative splicing controlled by hnRNP H/F regulates E-cadherin expression and hESC pluripotency

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We have discovered two errors in our published paper noted above. These errors, which were entirely textual, occurred in the Introduction and Discussion and in no way affect the results or conclusions of our study. We have corrected the errors as follows, and the updated version of our paper has replaced the previous version online.

First, we mistakenly incorporated two sentences in the first paragraph of the Introduction from an article by Venables et al. (2013). These two sentences have been deleted: “For example, different isoforms of Foxp1 produced from ESC-specific AS have differential effects on the induction of key pluripotency genes such as *OCT4* and *NANOG* (Gabut et al. 2011). Similarly, alternative splice forms of DNMT3B are specific to stem cells, implying that layered and integrated regulation of gene expression occurs at the levels of transcription and splicing (Gopalakrishna-Pillai and Iverson 2011).” This text has been replaced as follows: “For example, different isoforms of Foxp1 and Oct4, which are important transcription factors that function in determining stem cell identities, are produced by ESC-specific AS, and this controls their transcriptional activities and targets (Atlasi et al. 2008; Gabut et al. 2011). Similarly, isoforms of DNMT3B produced by AS are also known to be specific for stem cells, suggesting that AS regulation contributes to maintaining a stem cell-specific epigenetic state in ESCs (Gopalakrishna-Pillai and Iverson 2011; Liao et al. 2015).”

We sincerely apologize for this to both the authors of the previous paper and the readers of our article.

Second, in our study, we identified human embryonic stem cell (hESC)-specific alternative splicing (AS) regulation of transcripts encoded by the *TCF3* gene (also known as E2A) and established its functional significance during hESC differentiation. However, we were recently made aware that we had in some instances mistakenly referred to another gene, *TCF7L1*, as *TCF3* in both the Introduction and Discussion. We confused these two genes not only because *TCF7L1* has also frequently been referred to as *TCF3* but also because the encoded proteins have related functions and properties, including, as supported by our study, in hESC pluripotency and differentiation. We have corrected these errors as follows:

1. We used the incorrect name, “T-cell factor 3,” for TCF3 (E2A). The correct name is “transcription factor 3.” A sentence in the Abstract and one in the second paragraph of the Introduction have now been changed from “T-cell factor 3” to “transcription factor 3.”
2. We confused TCF3 (TCF7L1) with TCF3 (E2A) in several places in the Introduction and Discussion. We have now deleted references for TCF3 (TCF7L1) (see list of references for TCF7L1) and have corrected the inappropriate sentences regarding TCF3 (E2A) with additional, proper references (see list of additional references). The text changes are as follows:
 - a. Most of the paragraph at the bottom of p. 1161 and continued to the top of p. 1162 has been deleted and replaced as noted here:

Deleted: “More recent studies have revealed that TCF3 plays important roles in both stem cell maintenance and differentiation. In mouse ESCs (mESCs), ChIP-seq (chromatin immunoprecipitation [ChIP] combined with high-throughput sequencing) analysis revealed that TCF3 co-occupies the promoters of many of the genes regulated by Oct4, Sox2, and Nanog (Cole et al. 2008; Yi et al. 2008), countering the action of these factors and stimulating ESC differentiation (Pereira et al. 2006; Wray et al. 2011; Yi et al. 2011).et al. 2006; Wray et al. 2011; Yi et al. 2011). In fact, deletion of *TCF3* maintains high expression of pluripotency genes and delays mESC differentiation into three germ cell lines during embryoid body (EB) formation (Yi et al. 2008). Conversely, TCF3 is also able to repress differentiation-associated genes (Tam et al. 2008). In adult skin, TCF3 is expressed in epidermal stem cells located in the hair follicle bulge, activates a progenitor-associated expression program, and inhibits differentiation

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(Merrill et al. 2001; Nguyen et al. 2006). Thus, TCF3 is thought to be an important regulator of stem cell identity, capable of promoting either stem cell self-renewal or differentiation, depending on the cellular context."

Replacement text: "TCF3 is known to be involved in multiple developmental processes by functioning together with other HLH family proteins (Wang and Baker 2015; Miyazaki et al. 2017). Several studies have shown that TCF3 plays important roles in controlling maintenance and differentiation of tissue-specific adult stem cells. For example, TCF3 maintains the hematopoietic stem cell pool and promotes maturation of myelolymphoid and myeloerythroid progenitors (Semerád et al. 2009). TCF3 is also involved in controlling differentiation of neural stem cell into astrocytes (Bohrer et al. 2015). These reports imply that TCF3 functions to control either stem cell self-renewal or differentiation."

- b. On p. 1162, column 1, paragraph 2, this sentence has also been deleted: "However, possible functional differences between E12 and E47 have not been investigated in the context of stem cell maintenance."

The replacement text is: "However, the regulatory mechanisms by which E12 and E47 isoforms are produced and how this process might be regulated have not been investigated."

- c. On p. 1162, column 2, paragraph 1, this sentence has been deleted: "In this study, we focused on the AS changes affecting TCF3 because, as described above, TCF3 is a well-studied transcription factor that is known to be a key regulator of embryonic development."

The sentence above has been replaced as follows: "In this study, we focused on the AS changes affecting TCF3 because, as described above, TCF3 is already known to play important roles in various developmental pathways and we were therefore interested in elucidating its role and that of AS in hESCs."

- d. On p. 1168, column 2, paragraph 2, this sentence has been deleted: "Overexpression of TCF3 (E47) induces the EMT, transforming epithelial-like MDCK cells to a migratory phenotype by repressing *CDH1* transcription and stimulating mESC differentiation, although whether this activity is specific to E47 is not known (Pérez-Moreno et al. 2001; Yi et al. 2011)."

The replacement text for the above is: "Overexpression of TCF3 (E47) induces the EMT, transforming epithelial-like MDCK cells to a migratory phenotype by repressing *CDH1*, although whether this activity is specific to E47 is not known (Pérez-Moreno et al. 2001)."

- e. On p. 1169, column 2, paragraph 1, this text has also been deleted: "However, TCF3 was essentially rediscovered as an integral factor in the core regulatory circuitry of pluripotent stem cells, and multiple important roles have been reported, mostly in mESCs (see above; Pereira et al. 2006; Cole et al. 2008; Yi et al. 2008, 2011; Wray et al. 2011). Whereas E12 and E47 are now thought to function by differentially dimerizing with a variety of tissue specific class II HLH proteins (such as NeuroD1, Ets, Pax, and negative regulatory ID proteins) to control cell type specification and differentiation programs during embryonic development (Wang and Baker 2015 and references therein), possible functional differences between the two isoforms in stem cell maintenance had not been investigated previously."

The replacement text is: "In more recent studies, TCF3 has also been reported to play important roles in maintenance and differentiation of tissue-specific adult stem cells (see above). However, TCF3 functions in ESCs had not been investigated previously. Additionally, whereas E12 and E47 are now thought to function by differentially dimerizing with a variety of tissue-specific class II HLH proteins, such as NeuroD1, Ets, Pax, and negative regulatory ID proteins, to control cell type specification and differentiation programs during embryonic development (Wang and Baker 2015 and references therein), AS regulatory mechanisms by which the two isoforms are produced and possible functional differences between them in stem cells had not been investigated previously."

- f. On p. 1169, column 2, paragraph 2 has also been deleted: "TCF3 is well known to play important roles in controlling differentiation in mESCs. For example, knockdown of TCF3 in mESCs was shown to induce expression of *NANOG*, *OCT4*, and other pluripotency genes and result in a permanent undifferentiated state (Pereira et al. 2006; Cole et al. 2008). In light of this, it was unexpected when we found that knockdown of E12 and/or E47 did not result in induction of pluripotent genes in H9 hESCs. We suggest that this discrepancy arises from interspecies differences. There are, in fact, several substantial differences between hESCs and mESCs even though both are pluripotent cells derived from blastocyst embryos (Thomson et al. 1998; Sato et al. 2003). Most relevantly, significant interspecies differences in TCF3 and its target genes exist. For example, a recent study showed that TCF3 targets overlap less with those of the pluripotency regulators OCT4 and NANOG in hESCs than in mESCs and that human TCF3 acts generally on differentiation-specific rather than pluripotency-specific genes (Sierra et al. 2018). In addition, while we found that mutually exclusive AS of TCF3 switches exon 18a (E12) to exon 18b (E47) during hESC differentiation, Salomonis et al. (2010) reported that skipping of exon 4, which creates a short TCF3 isoform, is an mESC-specific AS event not conserved in hESCs even though the mESC-specific TCF3 isoform does regulate pluripotency and expression of early developmental genes. Furthermore, TCF3-responsive elements in the *CDH1* promoter are also different between humans and mice. In mice, TCF3 binds a palindromic E-box sequence, which is often recognized by homodimers

(Behrens et al. 1991; Pérez-Moreno et al. 2001), whereas multiple single E-box sequences are found in the human gene (Tiwari et al. 2015; Ahn et al. 2016). These observations suggest that regulation of TCF3 AS and TCF3 downstream targets such as *CDH1* may explain at least in part the interspecies differences between hESCs and mESCs. Consistent with this, cadherin/catenin-dependent intercellular attachment is crucial for survival of hESCs but not mESCs (Ohgushi et al. 2010)."

The replacement text is: "An important finding of our study is that TCF3 regulates expression of CDH1/E-cadherin, which is a fundamentally important molecule for determining hESC identity, and does so in an isoform-dependent manner. Interestingly, cadherin/catenin-dependent intercellular attachment is known to be crucial for survival of hESCs but not mESCs (Ohgushi et al. 2010). Consistent with this, TCF3-responsive elements in the *CDH1* promoter are different between humans and mice. In mice, TCF3 binds palindromic E-box sequences, which are often recognized by homodimers (Behrens et al. 1991; Pérez-Moreno et al. 2001), whereas multiple single E-box sequences are found in the human gene (Tiwari et al. 2015; Ahn et al. 2016). There are indeed several substantial differences between human and mouse ESCs even though both are pluripotent cells derived from the blastocyst embryo (Thomson et al. 1998; Sato et al. 2003). The above observations suggest that regulation of TCF3 AS and TCF3 downstream targets such as *CDH1* may explain at least in part the interspecies differences between human and mouse ESCs."

- g. On p. 1170, column 1, paragraph 2 has been deleted: "TCF3 is also known to play important roles in a variety of differentiation-related processes, but, in these cases, how AS might contribute is unknown. For example, in mice, TCF3 is highly expressed in neuronal stem cells of the subventricular zone niche, and *TCF3*-null embryos die at embryonic day 9.5 and display defects in neural and mesodermal patterning (Merrill et al. 2004). TCF3 also functions in skin stem cells to maintain an undifferentiated state and, through Wnt signaling, directs these cells along the hair lineage (Nguyen et al. 2006). It also has been reported recently that TCF3 coordinates early heart formation by functioning with other HLH and ID proteins (Cunningham et al. 2017). In addition to developmental functions, *TCF3* mutations have been reported to cause disease, such as Burkitt lymphoma (Schmitz et al. 2012). It will be of interest to determine whether any of these TCF3-dependent events are modulated by AS and, if so, whether changes in hnRNP H/F concentrations play a regulatory role."

The replacement text is: "Another important aspect of our study is our finding that TCF3 AS is regulated during hESC differentiation by hnRNP H/F. TCF3 is known to play important roles in a variety of differentiation-related processes, such as hematopoietic and neuronal development (Semerad et al. 2009; Bohrer et al. 2015). Moreover, it also has been reported recently that TCF3 coordinates early heart formation by functioning with other HLH and ID proteins (Cunningham et al. 2017). In addition to developmental functions, *TCF3* mutations have been reported to cause disease, such as Burkitt lymphoma (Schmitz et al. 2012). However, in all of the above cases, how AS might contribute is unknown. Thus, it will be of interest to determine whether any of these TCF3-dependent events are modulated by AS and, if so, whether changes in hnRNP H/F concentrations play a regulatory role."

List of deleted references

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We again apologize for our errors in the Introduction and Discussion sections of our paper. However, although we mistakenly referred to TCF3 (TCF7L1) in our study of TCF3 (E2A), this does not affect our conclusion that TCF3 (E2A) AS regulated by hnRNP H/F is important for controlling hESC maintenance and differentiation via a mechanism involving isoform-specific transcriptional control of *CDH1* expression.

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