Foreword to the Second Edition
Foreword to the First Edition
Preface to the First Edition
In remembrance of Keith Henry Stockman Campbell (23 May 1954–5 October 2012)
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In remembrance of Keith Henry Stockman Campbell (23 May 1954–5 October 2012)

Keith’s legacy survives as the work described in many chapters of this book was made possible by his contributions to the field of developmental biology. However, to many, he was much more than a colleague. He was a close and dear friend always with a smile on his face and the life of the party – of which there were many. Keith believed the best ideas occurred after hours and was able to convince many of us to join him in the pursuit. His natural curiosity led him to additional adventures outside the lab including sport fishing, shooting, whitewater rafting, scuba diving and fixing up old Volkswagens. He could also be a bit of an artist in the kitchen. While achieving high stature in science, Keith maintained a blue collar way about him, lacking pretention and always willing to share ideas. While serious about his work, he never took himself too seriously. It was easy to be his friend and colleague. Though he travelled much and could not always be with them, Keith’s daughters Lauren and Claire were always a topic of conversation. He was a proud father sharing their accomplishments and wanting the best for them. Keith’s life was perhaps the most enriched by his wife Kathryn Campbell, whose unwavering devotion and companionship was palpable when in their company. Keith’s legacy not only survives with the work represented in this Edition, but will continue to thrive with work that will be done by generations of scientists in years to come. New treatment tools for today’s untreatable diseases as well as a deeper understanding of basic biological processes will be possible only because of Keith’s scientific ingenuity, curiosity and search for truth was shared so generously with the rest of us.

This volume is dedicated to the lasting memory of Keith.
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Enhancing SCNT with Chromatin Remodeling Agents

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INTRODUCTION

Since it was first reported in 1997 that a sheep had been cloned (Wilmut et al., 1997), more than 10 additional mammalian species have been cloned successfully using somatic cell nuclear transfer (SCNT). The success of SCNT gives promise to applications such as species preservation, livestock propagation, and cell therapy for medical treatment by nuclear transfer embryonic stem cells (NT-ESCs) (Wakayama et al., 2001, 2005a). While cloning efficiencies can range from 0% to 20% when measured as offspring born healthy as a proportion of the total SCNT embryos transferred, efficiency rates of only 1–2% are typical for mice. These inefficiencies have limited such practical applications of SCNT. Moreover, many abnormalities in mice cloned from somatic cells have been reported, including abnormal gene expression in embryos (Boiani et al., 2002; Bortvin et al., 2003; Kishigami et al., 2006a), abnormal placenta (Wakayama and Yanagimachi, 1999; Tanaka et al., 2001), obesity (Tamashiro et al., 2000, 2002), and early death (Ogonuki et al., 2002). These have been major issues for SCNT technologies to overcome.

Many trials attempting to overcome the inefficiencies of SCNT have been reported, and these can mainly be divided into four types as follows.

Type 1: improvement by optimizing SCNT protocols in each condition for oocyte activation (Kishikawa et al., 1999; Terashita et al., 2012), timing of oocyte activation (Wakayama and Yanagimachi, 2001a), timing of enucleation or injection of nucleus (Wakayama et al., 2003), and culture medium SCNT embryos (Boiani et al., 2005; Campbell et al., 2007), as well as a serial cloning (Ono et al., 2001).

Type 2: improvement by modified donor cell preparation, including optimized cell cycle, chemical treatment of donor cells (Boiani et al., 2003; Wakayama and Yanagimachi, 2001b; Wakayama et al., 2005b).

Type 3: improvement by gene manipulation (Inoue et al., 2003; Wakayama and Yanagimachi, 2001b; Wakayama et al., 2005b).

Type 4: improvement by chemical treatment of reconstructed oocytes.
Although more or less significant improvement of SCNT has been reported in each type, the recent dramatic success achieved by treatment of reconstructed oocytes with histone deacetylase inhibitor (HDACi) has confirmed that SCNT can be improved at a practical level by using chemical agents.

**ESTABLISHMENT OF A CONCEPT FOR DIRECT TREATMENT OF SCNT EMBRYOS WITH CHEMICAL AGENTS**

The inefficiency of SCNT has been supposed to be attributed to epigenetic errors such as DNA methylation (Cibelli, 2007). Consistently, accumulating data have shown abnormal epigenetic modifications (Dean et al., 2001; Kang et al., 2001; Santos et al., 2003; Ohgane et al., 2004; Yamagata et al., 2007; Inoue et al., 2010), gene expression during pre- or post-implantation development (Humphreys et al., 2002; Suemizu et al., 2003; Inoue et al., 2006) and even after birth (Kohda et al., 2005), and chromosome segregation possibly by a particular epigenetic status of the centromere in SCNT (Mizutani et al., 2012). Therefore, it would be natural to aim at improvement of SCNT by correcting epigenetic modifications with chromatin remodeling agents.

The first success in direct treatment of SCNT embryos with chemical agents occurred accidentally in 2001, when 1% dimethyl sulfoxide (DMSO) in the activation medium was found to improve significantly the frequency of development to the blastocyst stage in vitro (Wakayama and Yanagimachi, 2001a), demonstrating that nuclear reprogramming can be enhanced artificially using chemical treatment, and establishing a concept of direct treatment of SCNT mouse embryos with chemical agents. Although the detailed mechanism underlying improvement by DMSO is unclear yet, DMSO treatment affects the DNA methylation status at multiple loci (Iwata et al., 2006), and also possibly improves SCNT through changing epigenetic modifications.

The first success in direct treatment of SCNT embryos themselves with chemical agents occurred accidentally in 2001, when 1% dimethyl sulfoxide (DMSO) in activation medium was found to improve significantly the frequency of development to the blastocyst stage in vitro.

**DISCOVERY OF THE OPTIMAL TREATMENT OF SCNT EMBRYOS WITH TRICHOSTATIN A**

Trichostatin A (TSA), a histone deacetylase inhibitor (HDACi), is representative of a chromatin remodeling agent that enhances the pool of acetylated histones (Yoshida et al., 1990) and DNA demethylation (Hattori et al., 2004). Enright et al. found that 0.08-μM TSA treatment of donor cells increased blastocyst development compared to controls (35.1% vs 25.1%; Enright et al., 2003). However, to date, chemical treatment of donor cells has shown minimal improvement in SCNT.

On the other hand, regardless of the report on DMSO as early as 2001, direct treatment of SCNT embryos with TSA was not instantly successful. In 2006, two groups independently discovered the optimum concentration, timing, and period of TSA treatment for cloned mouse embryos (Figure 11.1) (Kishigami et al., 2006b; Rybouchkin et al., 2006). Eventually this method led to a greater than five-fold increase (e.g., from 0.3% to 6.5%) in the success rate of mouse cloning (Figure 11.2; see also Table 11.1, below) and a doubling in the rate of establishing nES cell lines (Kishigami et al., 2006b). Based on these studies, the best protocol for TSA treatment in mice is: (1) reconstructed oocytes should be continuously exposed to TSA from the time point of oocyte activation for at least 10 h, but before the two-cell stage (Figure 11.3); (2) TSA concentrations of 5–50 nM are recommended, as TSA becomes effective from 5 nM but shows toxicity at 500 nM (Figure 11.4). Notably, the optimal time window for TSA treatment is narrow and specific, such as the first 10 h after oocyte activation. This finding reveals that this time period is crucial for success of reprogramming of SCNT embryos, which determines their developmental fate, at least in mice. Further, this time period corresponds to that before the initiation of zygotic gene activation (ZGA), implying that this enhancement of reprogramming by TSA is related to change of epigenetic state but is not directly coupled with transcriptional activity during this time period. TSA treatment for longer or shorter time periods, or even different timing, significantly reduces the effectiveness of TSA treatment on developmental potential. It should be also noted that TSA treatment enables around 40% of reconstructed oocytes to produce nES cells using B6D2F1 cumulus cells. Considering the establishment rate of normal ES cells from fertilized embryos, more than half of somatic cells should have the potential to be reprogrammed into nES cells, supporting the stochastic model for reprogramming rather than the elite model (Yamanaka, 2009), in which most or all cells have the potential to become at least pluripotent. Further, recently it has been demonstrated that the gene expression profile in cloned neonatal mice shows more normalization by TSA treatment; the total gene expression profile of the TSA clones resembles that of the pups born following fertilization by ICSI (Kohda et al., 2012). Thus, TSA treatment evidently enhances nuclear reprogramming to increase the success rate of cloning.
Chapter 11  Enhancing SCNT with Chromatin Remodeling Agents

**FIGURE 11.1** An experimental scheme of TSA treatment. According to the standard procedure of mouse cloning, donor nuclei from somatic cells are injected into enucleated oocytes. These reconstructed oocytes are activated by culture in the Ca^{2+}-free CZB medium including 5-mM Sr^{2+}, as well as TSA for TSA treatment except for the 14-h TSA treatment. After 6 h activation, the activation medium was changed to KSOM medium. For 10 h (or up to 20 h) of TSA treatment, activated oocytes are cultured in KSOM medium including TSA for another 4 h (another 14 h) and transferred into KSOM without TSA. These cloned embryos are cultured in KSOM until subject to embryo transfer into the surrogate mother (2nd day), examination of blastocyst formation (3rd or 4th day) or establishment for NT-ES cells (3rd or 4th day).

**FIGURE 11.2** Production of “normal” cloned mice after TSA treatment. TSA treatment often led to multiple conceptions. In one case, five fetuses in one foster were seen at full term (A). Produced cloned mice treated by TSA showed no obvious abnormalities with the exception of a large placenta (B), as also seen in “normal” clones without TSA treatment. (C) TSA treatment also led to the success of cloning ICR mice from adult cumulus cells. After cesarean section, pups showed normal appearance but had large placentas (C). All the pups were weaned normally and on time (D).
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<th>Development to Blastocyst (vs control)</th>
<th>Development to Term (vs control)</th>
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<td>50 nM</td>
<td>13</td>
<td>36% vs 30%</td>
<td>N.A.</td>
<td>No improvement</td>
<td>Iager et al., 2008</td>
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<tr>
<td>Mouse</td>
<td>Cumulus (B6D2F1)</td>
<td>TSA</td>
<td>5 nM</td>
<td>10</td>
<td>75% vs 23%</td>
<td>6.5% vs 0.3%</td>
<td>Kishigami et al., 2006b</td>
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<tr>
<td></td>
<td>Cumulus (ICR)</td>
<td>TSA</td>
<td>5 nM</td>
<td>10</td>
<td>42% vs 22%</td>
<td>6.5% vs 7.4% per blast</td>
<td>Sawai et al., 2012</td>
<td></td>
</tr>
<tr>
<td>Pig</td>
<td>Fibroblast</td>
<td>TSA</td>
<td>50 nM</td>
<td>24</td>
<td>46% vs 18%</td>
<td>N.A.</td>
<td>Live piglets produced</td>
<td>Li et al., 2008</td>
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<tr>
<td></td>
<td>Cumulus (C57BL/6)</td>
<td>SCR</td>
<td>250 nM</td>
<td>10</td>
<td>2.3% vs 0%</td>
<td>Better than TSA</td>
<td>Van Thuan et al., 2009</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C3He</td>
<td>SCR</td>
<td>250 nM</td>
<td>10</td>
<td>0.9% vs 0%</td>
<td>Better than TSA</td>
<td>Van Thuan et al., 2009</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DBA/2</td>
<td>SCR</td>
<td>250 nM</td>
<td>10</td>
<td>6.5% vs 0%</td>
<td>Better than TSA</td>
<td>Van Thuan et al., 2009</td>
<td></td>
</tr>
<tr>
<td></td>
<td>129/Sv</td>
<td>SCR</td>
<td>250 nM</td>
<td>10</td>
<td>9.8% vs 2.4%</td>
<td>Better than TSA</td>
<td>Van Thuan et al., 2009</td>
<td></td>
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</tr>
<tr>
<td>Pig</td>
<td>Cumulus (B6D2F1)</td>
<td>TSA +5-aza</td>
<td>100 nM + 10 nM</td>
<td>8</td>
<td>69% vs 69% (vs TSA only)</td>
<td>1% vs 13% (vs TSA only) at E10.5</td>
<td>Tsuji et al., 2009</td>
<td></td>
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</tr>
<tr>
<td>Pig</td>
<td>Cumulus (B6D2F1)</td>
<td>SAHA</td>
<td>1.0 μM</td>
<td>10</td>
<td>70% vs 31%</td>
<td>9.4% vs 2.6%</td>
<td>Ono et al., 2010</td>
<td></td>
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</tr>
<tr>
<td>Pig</td>
<td>Cumulus (B6D2F1)</td>
<td>VPA</td>
<td>2 mM</td>
<td>10</td>
<td>57% vs 51%</td>
<td>8% vs 7% (BD129F1)</td>
<td>No effect Ono et al., 2010</td>
<td></td>
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<tr>
<td>Pig</td>
<td>Cumulus (B6D2F1)</td>
<td>Ox</td>
<td>1.0 μM</td>
<td>10</td>
<td>63% vs 50%</td>
<td>7.5% vs 2.6%</td>
<td>Ono et al., 2010</td>
<td></td>
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</tr>
<tr>
<td>Pig</td>
<td>Cumulus (B6D2F1)</td>
<td>CBHA</td>
<td>20 μM</td>
<td>10</td>
<td>70% vs 33%</td>
<td>3.6% vs 0.8%</td>
<td>Dai et al., 2010</td>
<td></td>
</tr>
<tr>
<td>Pig</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Pig</td>
<td>Fibroblast</td>
<td>TSA</td>
<td>37.5 nM</td>
<td>24</td>
<td>81% vs 54%</td>
<td>N.A.</td>
<td></td>
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<td>Pig</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td>Cell Type</td>
<td>Treatment</td>
<td>Concentration</td>
<td>Time</td>
<td>Effects</td>
<td>Reference</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Fibroblast (NIH miniature pig)</td>
<td>SCR</td>
<td></td>
<td>500 nM</td>
<td>14–16</td>
<td>21% vs 9%</td>
<td>Zhao et al., 2009</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TSA SCR</td>
<td></td>
<td>50 nM 500 nM</td>
<td>10</td>
<td>23% vs 10% 25% vs 11% 0.8% vs 0.4% 1.6% vs 0.4%</td>
<td>Zhao et al., 2010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibroblast Bone marrow</td>
<td>TSA</td>
<td></td>
<td>10 ng/ml</td>
<td>10</td>
<td>45% vs 24% 30% vs 27% 11% vs 10% 5% vs 0%</td>
<td>Dependence on the nuclear cell type</td>
<td>Lee et al., 2010</td>
<td></td>
</tr>
<tr>
<td>Fibroblast</td>
<td>VPA</td>
<td></td>
<td>5 mM</td>
<td>24</td>
<td>41% vs 23%</td>
<td>Increasing ICM cell no.</td>
<td>Kim et al., 2011</td>
<td></td>
</tr>
<tr>
<td>Fibroblast</td>
<td>TSA</td>
<td></td>
<td>50 nM</td>
<td>24</td>
<td>30% vs 15%</td>
<td>11% vs 0% NT–ES lines</td>
<td>Vassiliev et al., 2011</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>Fibroblast</td>
<td>TSA</td>
<td>100 nM</td>
<td>6</td>
<td>45% vs 23%</td>
<td>Shi et al., 2008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumulus (Hycole hybrid)</td>
<td>TSA</td>
<td></td>
<td>5 nM</td>
<td>10</td>
<td>79% vs 80% 5% vs 3%</td>
<td>No adulthood after TSA treatment</td>
<td>Meng et al., 2009</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>Fibroblast (SD and Wistar)</td>
<td>TSA</td>
<td>50 nM</td>
<td>10–14</td>
<td>N.A.</td>
<td>0% vs 0%</td>
<td>Sterthaus et al., 2009</td>
<td></td>
</tr>
<tr>
<td>Inter-species (oocyte donor)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Rabbit–Human</td>
<td>Fibroblast</td>
<td>TSA</td>
<td>100 nM</td>
<td>3</td>
<td>5% vs 2%</td>
<td>Shi et al., 2008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black-footed cat–Domestic cat</td>
<td>Fibroblast</td>
<td>TSA</td>
<td>50 nM</td>
<td>20</td>
<td>3.3% vs 3.3% 0% vs 0%</td>
<td>Gomez et al., 2011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long-tailed macaque-pig</td>
<td>Fibroblast</td>
<td>TSA</td>
<td>10 nM</td>
<td>48</td>
<td>23% vs 10%</td>
<td>Qin et al., 2012</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TSA, trichostatin A; SCR, Scriptaid; SAHA, suberoylanilide hydroxamic acid; VPA, valproic acid; Ox, Oxamflatin; 5-aza, 5-aza-2’-deoxycytidine; CBHA, m-carboxycinnamic acid bis-hydroxamide.

*The following species were studied: cow, mouse, pig, rabbit, and rat.
Can TSA treatment of animal cloning be used for any type of cells in mice? Significant improvement, to a greater or lesser degree, has been observed in cloning from any somatic cells, including cumulus, fibroblast, spleen, and neural stem cells, in mice. However, so far no success has been reported in improving ES-cell cloning by TSA treatment; rather, it appears to sabotage development (Kishigami et al., 2006b). The genomes of ES cells should already have an optimal epigenetic state at a reduced DNA methylation state which may be optimal for cloning. Therefore, TSA treatment in ES cloning may be toxic, as is high-dose TSA treatment in somatic cloning. In fact, TSA treatment following somatic cloning also led to a success rate of 6–7%, which is comparable to that of ES cloning (~5%) (Wakayama et al., 1999). Therefore, it may be possible to interpret that TSA treatment of somatic cloning brings transferred somatic nuclei close to ES-like nuclei.

### SCNT USING HDACi TREATMENT

Before the discovery of TSA treatment, most cloned mice were limited to hybrid strains and had never been cloned from outbred or inbred strains (Wakayama and Yanagimachi, 2001b; Inoue et al., 2003). Following the establishment of optimal TSA treatment conditions, it was found that TSA treatment was applied for producing cloned mice even from an outbred, supposedly “unclonable,” strain (Kishigami et al., 2007), demonstrating that apparent “unclonability” of certain mouse strains does not indicate that nuclear reprogramming of those nuclei is impossible. Regardless, most of the important mouse strains have still not been cloned successfully. Subsequently, Scriptaid, another HDACi, was found to increase cloned embryo development not only in hybrid but also in inbred strains, and this allowed us to generate full-term offspring from several inbred mouse strains, such as C57BL/6 and C3H/He (Figure 11.5; Table 11.1) (Van Thuan et al., 2009).

Although TSA application resulted in great improvements in SCNT cloning in mice, the effects of TSA treatment on cloning efficiency in other species are still controversial.

Although TSA application resulted in great improvements in SCNT cloning in mice, the effects of TSA treatment on cloning efficiency have been controversial in cow (Iager et al., 2008; Wu et al., 2008), pig (Li et al., 2008;
Yamanaka et al., 2009), rabbit (Shi et al., 2008; Meng et al., 2009), and rat (Sterthaus et al., 2009) (Table 11.1). Moreover, some groups have reported that TSA treatment had detrimental effects on the in vitro and in vivo development of the SCNT embryos (Wu et al., 2008; Meng et al., 2009). In experiments on rabbits, all cloned offspring treated with TSA died within 19 days of birth, whereas the untreated control clones grew to adulthood (Meng et al., 2008). On the other hand, it is known that the drug Scriptaid acts as an HDACi but is less toxic than TSA (Su et al., 2009). Using this drug, Zhao and colleagues managed to improve the success rate of pig cloning to full term (Zhao et al., 2009). To our knowledge, the effects of TSA treatment on full-term development have not been determined in any species other than the mouse. These results suggest that although the use of HDACi drugs can enhance reprogramming in cloned embryos, their toxicity means that the effects depend on the sensitivity of the donor cell type, strain, or species.

THE POSSIBLE MECHANISM UNDERLYING HDACi TREATMENT TO ENHANCE NUCLEAR REPROGRAMMING

Although the underlying mechanism of how HDACi treatment improves cloning efficiency remains unknown, it is thought that it can induce hyperacetylation of the core histones, resulting in structural changes in chromatin that permit transcription and enhanced DNA demethylation of the somatic cell-derived genome following SCNT (Kishigami et al., 2006b), which is a necessary part of genetic reprogramming (Simonsson and Gurdon, 2004). In fact, several reports have clearly shown that HDACi treatment improved histone acetylation (Wang et al., 2007; Yamanaka et al., 2009), nascent mRNA production (Van Thuan et al., 2009), and gene expression (Tsuji et al., 2009) in a manner similar to that in normally fertilized embryos.

However, how histone methylation is modified in TSA-treated cloned embryos is not completely understood. Recently, TSA treatment was found to cause an increase in chromosome decondensation and nuclear volume in SCNT-generated embryos, similar to embryos produced by intracytoplasmic sperm injection (ICSI) (Bui et al., 2010). Histone acetylation was increased in parallel with chromosome decondensation. This was associated with a more effective formation of DNA replication complexes in treated embryos. Interestingly, the proportion of SCNT-generated embryos showing an asymmetric expression of nascent RNA between blastomeres was significantly reduced in the TSA-treated group compared with controls at the two-cell stage. These results suggest that the incomplete and inaccurate genome reprogramming of SCNT-generated embryos was improved by TSA treatment.

THE TARGETS OF HDACi TO ENHANCE NUCLEAR REPROGRAMMING

In general, the HDAC enzymes are divided into five categories: class I (HDAC1, 3, and 8), class IIa (HDAC4, 5, 7, and 9), class IIb (HDAC6 and 10), class III (SIRT 1–7), and class IV (HDAC11) (Blackwell et al., 2008). As shown in Table 11.1, so far seven classes of HDACi have been examined in our laboratory. TSA, Scriptaid, SAHA, and oxamflatin are inhibitors for class I and IIa/b HDACs (Marks et al., 2003; Zhang and Dent, 2005; Mukhopadhyay et al., 2006; Blackwell et al., 2008; Chuang et al., 2009; Codd et al., 2009; Kuhn et al., 2009). These significantly improved cloning efficiency. On the other hand, APHA is an inhibitor of class I and IIa/b HDACs, but is greater than 10-fold more active against HDAC3 (class I) and HDAC6 (class IIb) than the other HDACs (Mai et al., 2003; Blackwell et al., 2008). VPA is an inhibitor for class I and IIa HDACs (Chuang et al., 2009). However, these two drugs did not improve the mouse cloning success rate (Figure 11.6) (Ono et al., 2010).

Sirtinol is an inhibitor of class III HDAC, but its effect on cloning was very limited (Hirata, 2008). Thus, inhibition of class IIb HDACs (HDAC6 and 10, but most likely HDAC10) appears to be very important for improving the success rate in cloning mice (Table 11.2). It is known that the class IIb HDACs preferentially not only target nuclear histone deacetylase but also play important roles in the regulation of heat shock protein (HSP)-mediated vascular endothelial growth factor receptors (Park et al., 2008). These other pathways could have negative effects on embryo development or on complete genomic reprogramming following SCNT.
TABLE 11.2 Summary of Improvements in Mouse Cloning Outcome and Characteristics of Each Histone Deacetylase Inhibitor (HDACi)

<table>
<thead>
<tr>
<th>Class of HDAC</th>
<th>Type of HDAC</th>
<th>TSA</th>
<th>SCR</th>
<th>SAHA</th>
<th>Ox</th>
<th>APHA</th>
<th>VPA</th>
<th>SIRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>HDAC 1, 2, and 8</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>○</td>
<td>●</td>
<td>×</td>
</tr>
<tr>
<td>Class I</td>
<td>HDAC 3</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>×</td>
</tr>
<tr>
<td>Class IIa</td>
<td>HDAC 4, 5, 7, and 9</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>○</td>
<td>●</td>
<td>×</td>
</tr>
<tr>
<td>Class IIb</td>
<td>HDAC 6</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>×</td>
</tr>
<tr>
<td>Class IIb</td>
<td>HDAC 10</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>○</td>
<td>●</td>
<td>×</td>
</tr>
<tr>
<td>Class III</td>
<td>SIRT 1–7</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>●</td>
</tr>
<tr>
<td>Improvement in cloning?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Slight</td>
<td></td>
</tr>
<tr>
<td>Reference*</td>
<td>1, 3, 4</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

TSA, trichostatin A; SCR, Scriptaid; SAHA, suberoylanilide hydroxamic acid; Ox, oxamflatin; VPA, valproic acid; APHA, aroyl pyrrolyl hydroxamide; SIRT, sirtinol; UP, unpublished observation; ●, inhibit normally; ○, 10-fold lower; ×, no effect.

*1, Kishigami et al. (2006b); 2, Van Thuan et al. (2009); 3, Ono et al. (2010); 4, Hirata (2008).

WHY DO CLONED EMBRYOS REQUIRE HDACi TREATMENT FOR BETTER GENOMIC REPROGRAMMING?

In nature, the oocyte cytoplasm contains reprogramming mechanisms, such as histone acetylation or DNA demethylation, that convert the sperm and oocyte nuclei to a totipotent state (Mayer et al., 2000; Wang et al., 2007; Feil, 2009). However, it is not yet clear whether these reprogramming factors are sufficient to reprogram the somatic cell nucleus, because the potential reprogramming machinery of the oocyte cytoplasm is prepared for the receipt of a gametic nucleus, not a somatic cell nucleus. In general, it is considered that the incomplete reprogramming of somatic
cell nuclei following SCNT arises from poor reprogramming in the oocyte. However, we now think that the oocyte cytoplasm might reprogram the somatic cell nucleus too strongly, or that the somatic cell nucleus is more sensitive to oocyte reprogramming factors than is the gametic cell nucleus. Therefore, by inhibiting a particular HDAC – especially a class IIb HDAC – during reprogramming, the donor nuclei in our studies were possibly reprogrammed more correctly (Kishigami et al., 2006b; Van Thuan et al., 2009), resulting in a higher success rate for cloning.

**CONCLUDING REMARKS**

The advent of cloning from adult-derived cells in 1997 marked a new departure in the study of key biological problems in NT biology (Wilmut et al., 1997), finally developing iPS cells technology in just one decade. Thus, reprogramming technologies have evolved rapidly. However, the reprogramming mechanism underlying SCNT still remains largely unclear after more than 15 years. The success in significant improvement of SCNT by HDACi has provided many clues in understanding the reason for the inefficiency of SCNT. Future study should be focused on identifying target HDACs of HDACis, as well as the target proteins of HDACs and their roles of embryonic development. Literature regarding successful improvement of SCNT development using agents other than HDACi is still limited. Further, this type of approach to find chemical agents to enhance reprogramming in SCNT could lead to understanding of the reprogramming mechanism in SCNT, thus making SCNT a truly practical tool.

**REFERENCES**


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5-aza-2′-deoxycytidine and trichostatin A. Theriogenology 70 (4), 622–630.


