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Review

Intrinsic and extrinsic molecular determinants or modulators for epigenetic remodeling and reprogramming of somatic cell-derived genome in mammalian nuclear-transferred oocytes and resultant embryos

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Abstract

The efficiency of somatic cell cloning in mammals remains disappointingly low. Incomplete and aberrant reprogramming of epigenetic memory of somatic cell nuclei in preimplantation nuclear-transferred (NT) embryos is one of the most important factors that limit the cloning effectiveness. The extent of epigenetic genome-wide alterations, involving histone or DNA methylation and histone deacetylation, that are mediated by histone-lysine methyltransferases (HMTs) or DNA methyltransferases (DNMTs) and histone deacetylases (HDACs) can be modulated/reversed via exogenous inhibitors of these enzymes throughout *in vitro* culture of nuclear donor cells, nuclear recipient oocytes and/or cloned embryos. The use of the artificial modifiers of epigenomically-conditioned gene expression leads to inhibition of both chromatin condensation and transcriptional silencing the genomic DNA of somatic cells that provide a source of nuclear donors for reconstruction of enucleated oocytes and generation of cloned embryos. The onset of chromatin decondensation and gene transcriptional activity is evoked both through specific/selective inactivating HMTs by BIX-01294 and through non-specific/non-selective blocking the activity of either DNMTs by 5-aza-2'-deoxycytidine, zebularine, *S*-adenosylhomocysteine or HDACs by trichostatin A, valproic acid, scriptaid, oxamflatin, sodium butyrate, *m*-carboxycinnamic acid *bis*hydroxamide, panobinostat, abexinostat, quisinostat, dacinostat, belinostat and psammaplin A. Epigenomic modulation of nuclear donor cells, nuclear recipient cells and/or cloned embryos may facilitate and accelerate the reprogrammability for gene expression of donor cell nuclei that have been transplanted into a host ooplasm and subsequently underwent dedifferentiating and re-establishing the epigenetically dependent status of their transcriptional activity during pre- and postimplantation development of NT embryos. Nevertheless, a comprehensive additional work is necessary to determine whether failures in the early-stage reprogramming of somatic cell-inherited genome are magnified downstream in development of cloned conceptuses and neonates.

Key words: somatic cell nucleus, reconstructed oocyte, epigenomic maturity, nuclear-transferred embryo, somatic cell-inherited chromatin remodeling, donor nuclear reprogramming, epigenetic modulator/modifier

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Species-specific differences in the reprogramming status of donor nuclear DNA epigenomic inheritance between mammalian somatic cell cloned embryos

Transcriptional activity (gene expression or repression) of the somatic cell genome during pre- and postimplantation development of cloned embryos depends generally on the reprogramming extent of epigenetic modifications such as demethylation/methylation of nuclear DNA cytosine residues as well as acetylation/deacetylation and demethylation/methylation of lysine moieties within chromatin nucleosomal core-derived histones H3 and H4. It has been ascertained that the donor genomic DNA should undergo both the global and developmentally-important gene-selective demethylation processes throughout the early embryogenesis of nuclear transfer-derived oocytes to reset its own epigenetic memory established as a result of specific differentiation pathway of somatic and germ cell lineages (Martinez-Diaz et al. 2010, Buganim et al. 2013, Masala et al. 2017). Against a background of other mammalian species zygotes that undergo active male pronuclear demethylation wave, in rabbits, both the paternally- and maternally-inherited genomes appear to maintain the relatively high DNA methylation level during preimplantation development up to the 16-cell stage. Admittedly, the correlation between the frequency of nuclear DNA demethylation and advanced degree of chromatin architectural remodelling has been suggested (Shi et al. 2004, Yang et al. 2007, Nashun et al. 2015). Nevertheless, this dependence is presumably insufficient, because the sperm-derived genome (male pronucleus) in non-mammalian species undergoes the spatial rearrangements of chromatin nucleosomal conformation without active demethylation of DNA cytosine residues. Although active (replication-independent) demethylation of nuclear genome seems to be preserved by the embryos of different mammalian species, in the rabbit embryos high DNA methylation levels were found to be maintained during preimplantation development (Shi et al. 2004, Corry et al. 2009, Shi and Wu 2009). In contrast, Lepikhov et al. (2008) have shown that, in rabbit nuclear-transferred embryos at the 1-cell stage, the fibroblast cell-inherited genomic DNA undergoes rapid demethylation immediately after activation of reconstituted oocyte (clonal cybrid) and formation of pseudopronucleus. Nonetheless, in ovine embryos derived from fertilized ova, a dramatic decrease in genomic DNA methylation status was not found until the 16-blastomere stage will not have been reached by them (Beaujean et al. 2004, Wen et al. 2014, Jafarpour et al. 2017).

In the recipient cell's cytoplasmic environment of the majority of mammalian species embryos, excluding sheep, heavily methylated somatic cell-inherited genome has to undergo wide epigenetic changes, which allow to erase its own methylation pattern established during cell differentiation and to restore nuclear totipotency/pluripotency during early embryogenesis. The studies aimed at examining development of murine preimplantation embryos (Kishigami et al. 2006, Esteves et al. 2011, Mason et al. 2012) have shown not only differential (asymmetric) demethylation waves of nucleosomal histones of topologically-separated parental genomes that had been previously configured into female and male pronuclei in the fertilized eggs, but also genome-wide DNA methylation changes during preimplantation development. It cannot also be excluded that zygotic demethylation and genome-wide methylation changes are not a prerequisite for normal development of ovine embryos, in which a global DNA demethylation wave does not occur immediately after fertilization of ova. Despite incomplete and delayed demethylation processes within somatic genome that had been transferred into the artificially activated eggs, some mammalian cloned embryos developed apparently normally (Rodriguez-Osorio et al. 2012, Anckaert and Fair 2015, Huang et al. 2016).

Influence of oocyte reconstruction technique (somatic cell nuclear transfer/SCNT method) on the fate of the processes for architectural remodeling and epigenetic reprogramming of donor genome in cloned embryos

Nucleoplasmic (karyolymphatic) factors of a somatic cell that are engaged directly or indirectly in its structural and functional differentiation are associated with nuclear chromatin and their qualitative and quantitative composition undergoes changes together with progressing cytodifferentiation state (Campbell and Alberio 2003, Eilertsen et al. 2007, Fisher and Fisher 2011). The previously-mentioned nucleoplasmic factors involve among others transcription factors, histones, non-histone HMG (high mobility group) proteins interacting with transcriptionally-active chromatin, nuclear lamins and poly-subunit protein complexes that are responsible for remodeling of spatial conformation of chromatin structures and for DNA topology changes. These latter include, e.g., nucleosome remodeling factor (NURF), *brahma* family proteins (BRG1 and BRM) sharing homology with related yeast factors SWI2/SNF2 (switch of mating type/sucrose non-fermenting) and multimeric epigenetic modifiers, the members of which are: tran-

scriptional activator complexes such as Trithorax group (Trx-G) proteins and transcriptional repressor complexes such as Polycomb group (Pc-G) proteins (Andreu-Vieyra and Matzuk 2007, Rajasekhar and Begemann 2007, Whitworth and Prather 2010, Buganim et al. 2013). When G0/G1-stage or G2/M-stage whole donor cell is fused with enucleated oocyte by electroporation or microinjected directly into the ooplast cytoplasm, then those specific factors of a somatic cell are also transferred into the cytoplasm of recipient oocyte and may block an ability of endogenous oocyte factors for appropriate remodeling/reprogramming of both epigenetic and genomic imprinting memory in foreign (allogenic) cell nucleus (Armstrong et al. 2006). Exogenous cytoplasmic factors of donor cell are incorporated together with own proteins and maternal transcripts (mRNA molecules) of oocyte into the remodeled somatic cell nucleus (pseudopronucleus), after its formation in a consequence of activating reconstructed (SCNT-derived) oocyte (Prather et al. 2009, Narbonne et al. 2012, Hörmanseder et al. 2017). In turn, an overabundance of these hypothetical foreign agents in the ooplasm causes a considerable dilution of specific internal oocyte factors (due to bilateral resuspending/mixing in the hybrid cytoplasmic environment), diminishing simultaneously the probability of complete donor nucleus reprogramming (Reik 2007, Van Thuan et al. 2009, Yan et al. 2010). On the contrary, the chief purpose of intraooplasmic microinjection of G0/G1-phase karyoplast-mediated somatic cell nucleus is to avoid all the above-mentioned problems related to the biochemical/molecular processes that occur in the nuclear-cytoplasmic hybrid (cybrid)-descended cells of preimplantation cloned embryos immediately after electrically-induced fusion of G0/G1- or G2/M-stage somatic cell-ooplast couplets. These problems at the molecular level can also take place in mammalian cloned embryos generated by direct microinjection of quiescent or cycling whole cells into the cytoplasm of enucleated oocytes (Roh and Hwang 2002, Kawano et al. 2004, Esteves et al. 2011). Introduction of practically only the donor cell nucleus at the G0/G1 or G2/M phases of mitotic cycle into the cytoplasm of enucleated oocyte increases many times the probability of proper action of specific cytosolic oocyte agents on the processes of foreign nuclear chromatin remodeling and genome reprogramming, because in this case the only source of exogenous proteins and mRNA transcripts is the nucleoplasm of transplanted karyoplast. Insignificant numbers of perinuclear cytoplasm (perikaryon) remain presumably without a greater effect on the further embryonic development of mammalian clonal zygotes (Galli et al. 2002, Kurome et al. 2003, Lee et al. 2003, Hiendleder 2007). Moreover, reducing the volume of allogeneic somatic cell-derived cytoplasm, which is transplanted into the cytosolic microenvironment of ooplast, allows to completely prevent the limitations caused by the hybridization of heteroplasmic sources of not only mitochondrial DNA (mtDNA) copies (Fig. 1), but also cytoplasmic and intramitochondrial translation system-descended messenger RNAs (including also polycistronic mitochondrial mRNA fractions), transporter RNAs as well as ribosomal RNAs. All these mtDNA or RNA fractions of heteroplasmic origin are inherited both from nuclear donor somatic cell and from nuclear recipient cytoplast (ooplast) (Roh and Hwang 2002, Bowles et al. 2007, Jin et al. 2017a). The lack of the impurities in the form of somatic cell-inherited mtDNAs in the cytoplasmic environment of reconstructed oocyte, or the lack of the so-called mtDNA heteroplasmy (Fig. 1) brings about a consequent decrease in the frequency of the disorders in the epigenetic reprogramming of nuclear DNA and mtDNA (due to hypermethylation or excessive demethylation of DNA cytosine residues) (Burgstaller et al. 2007, Whitworth and Prather 2010, Mallol et al. 2014, 2016). For those reasons, all the disturbances in dynamic homeostasis of epigenetic modifications of somatic cell genome may result from asynchronous structural remodeling of nuclear chromatin and thereby non-coordinated deacetylation/acetylation of histones and elevation of nucleosomal repression level through decrease of SWI2/SNF2 protein complex activity (Kumar et al. 2007, 2013, Liang et al. 2015, Jin et al. 2017b). They may also be triggered by asynchronous changes of spatial configuration of regulatory segments in the so-called displacement loop (D-loop) of naked' circular mtDNA molecules within the blastomeres of nuclear-transferred embryos (Hiendleder 2007, Zhao et al. 2010a, Srirattana et al. 2011, Narbonne et al. 2012). The maintenance of correct DNA methylation pattern in the cell nuclei of all descendant blastomeres of preimplantation cloned embryos favors also the preservation in the intact form of the mechanisms responsible for parental genome imprinting, i.e., uniparental/monoallelic gene expression. In turn, this is reflected in proper rearrangement of exogenous chromatin as well as faithful reprogramming of nuclear and mitochondrial (cytoplasmic) genetic apparatuses inherited from the somatic cell. But, in the extreme cases, this is even accompanied by partial remodeling of nuclear donor cell-derived chromatin structures, which enables avoiding the inhibition of transcriptional activity of a larger part of embryonic genome in the early stages of cloned embryo development (Yan et al. 2011, Saini et al. 2014, Nashun et al. 2015).

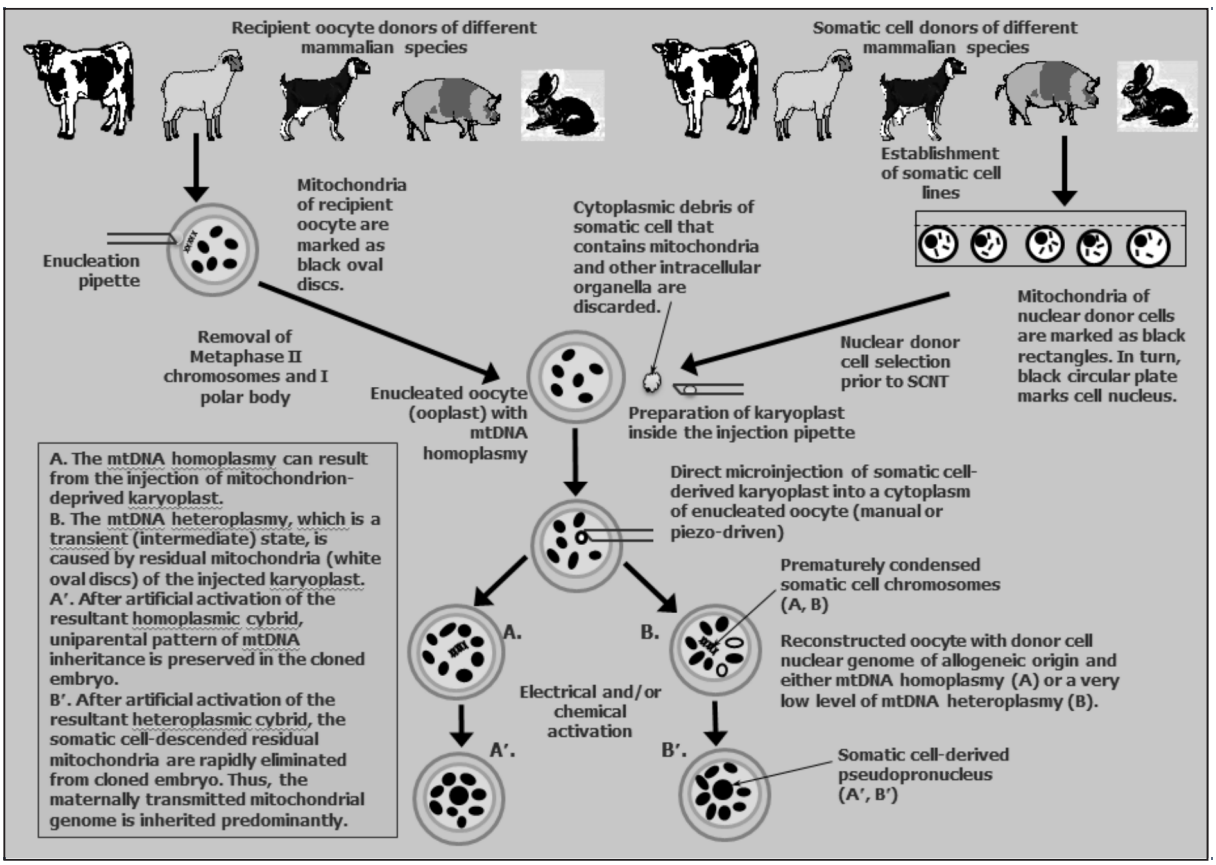

Fig. 1. Distribution of donor cell-inherited mtDNAs in nuclear-transferred oocytes reconstructed by intracytoplasmic microinjection of somatic cell-derived karyoplasts. By using the pipette, whose sharp bevelled tip has an external diameter about half the size of the selected cell, the plasma membrane is broken by gentle repeated aspiration of the entire cell into and out of the pipette. Thus, the cell nucleus with residual, perinuclear protoplasmic "ring" (i.e., perikaryon) is isolated. This live-membrane structure that has been obtained by mechanically induced lysis of the somatic cell is designated as a karyoplast. The karyoplast can also contain low numbers of somatic cell-derived mitochondria within the perikaryon. Therefore, the proportion of nuclear donor mtDNA copies in the clonal cytoplasmic hybrids (cybrids) seems to be related to the quantity of somatic cell cytoplasm present post reconstruction of enucleated oocytes.

Endogenous and exogenous factors responsible for architectural remodeling and epigenetic remodeling/reprogramming of somatic cell-inherited chromatin in a cytoplasm of nuclear-transferred oocytes

The establishment of epigenomic maturity in nuclear recipient oocytes

The remodeling and reprogramming of somatic cell-derived nuclear apparatus in cloned embryos is a result of interaction of protein factors accumulated in the nucleoplasm and attached to the chromatin, configured in the form of metaphase plate in consequence of appropriate rearrangement of its spatial structure and nucleosome repression, with protein factors of recipient oocyte cytoplasm (i.e., host ooplasm). Both former and latter protein factors, whose concentration and activity at the high levels are the prerequisites for establishment of the state of cytoplasmic, nuclear and epigenomic maturity of enucleated host oocyte (ooplast/cytoplast) for somatic cell-inherited nuclear genome, involve many pathways of intracellular enzymatic machinery. The most important protein members of this machinery are cyclin-dependent kinases (CDKs). At the metaphase II (MII) stage of the meiotic cell cycle, they include, among others, maturation/meiosis-promoting factor (MPF) and cascade of mitogen-activated protein kinases (C-MAPKs) related to the activity of cytostatic factor (CSF). MPF is a heterodimeric enzyme complex that consists of the catalytic subunit ($p34^{cdc2}$/CDK1; 34-kDa cell division control protein kinase 2/cyclin-dependent protein kinase 1) and the regulatory subunit (cyclin B). Furthermore, at the anaphase II (AII) stage, the protein factors regulating the oocyte meiotic division cycle include, e.g., poly-subunit protein complex of ubiquitin ligase that was named anaphase-promoting complex or cyclosome (APC/C) (Lee and Campbell 2006, Prather et al.

2009). Aside from determination of cytoplasmic and nuclear (meiotic) maturity of recipient oocytes, the above-mentioned CDKs bias the architectural remodeling of oocyte-descended microtubule organizing centers (MTOCs) or, more precisely, its acentriolar meiotic spindle poles (astrospheres). Additionally, they impact the spatial remodeling of both nuclear donor somatic cell-inherited chromatin and MTOCs (i.e., dicentriolar centrosomes or tetracentriolar diplosomes) that have been transplanted into host ooplasm (Fissore et al. 1999, Campbell and Alberio 2003, Ito et al. 2004). In turn, the presence of intrinsic epigenetic determinants and/or modifiers has been found to be indispensable both for attaining epigenomic maturity by MII-stage oocytes and for acquiring epigenomic competence by nuclear-transferred (NT) oocytes that have been artificially activated to induce their release from MII arrest and meiosis resumption. The crucial representatives of these endogenous epigenetic factors and modulatory proteins are: 1) competitive inhibitors of DNA methyltransferases/methylases (inhibitors of DNMTs; iDNMTs) such as isosteric blockers of the DNMT1o and DNMT3a/3b isoenzymes; 2) repressors of activity of methyl-CpG-binding proteins (MeCPs; proteins binding methylated 5'-cytidine-3'-monophosphate-5'-guanosine-3'/CpG dinucleotides/motifs); and 3) histone H3 and H4 acetyltransferases/acetylases (HATs) (Beaujean et al. 2004, Bonk et al. 2008, Das et al. 2010, Rodriguez-Osorio et al. 2012, Masala et al. 2017). The other pivotal intrinsic epigenetic modulators, which are accumulated in the cytoplasm and nucleoplasm of NT oocytes stimulated to initiate embryonic development, encompass: 4) isosteric inhibitors of histone deacetylases (HDACs); 5) supressory proteins of histone methyltransferases (HMTs); 6) histone demethylases/deiminases (HDMs); as well as 7) multi-subunit protein complexes with the activity of ATPases such as chromatin remodeling complexes (ChRs) (Yamanaka et al. 2009, Zhao et al. 2009, 2010a, Whitworth and Prather 2010, Nashun et al. 2015, Anckaert and Fair 2015, Hörmanseder et al. 2017).

The use of ectopic repressory proteins for selective/specific or non-selective/non-specific CDK inhibition (e.g., *R*-roscovitine, butyrolactone I or 6-dimethylaminopurine) and/or exogenous epigenetic modifiers (e.g., non-specific inhibitors of DNMTs or inhibitors of HDACs) throughout *in vitro* maturation of mammalian dictyotene- or germinal vesicle (GV)-stage oocytes can affect, on the one hand, the ability of the oocytes that reached MII stage to attain the cytoplasmic and epigenomic maturity states (i.e., before or simultaneously with acquisition of the nuclear/meiotic maturity by them). On the other hand, both the timing and rate of the cytoplasmic and epigenomic maturation as well as the degree and rapidity of synchronization between cytoplasmic, epigenomic and nuclear (meiotic) maturation can be affected by the treatment of immature (GV-stage) oocytes with the above-mentioned agents. Moreover, the developmental competences of somatic cell cloned embryos derived from nuclear recipient oocytes that have been matured *in vitro* in such conditions can also be influenced, to a high degree, by the modulators of cytoplasmic and epigenomic *ex vivo* maturation (Coy et al. 2005, Schoevers et al. 2005, Kishigami et al. 2006, Bui et al. 2007, Samiec and Skrzyszowska 2012, Liang et al. 2015, Xie et al. 2016).

Epigenomic modulation (epigenetic transformation) of nuclear donor somatic cells, nuclear recipient oocytes and/or *in vitro* cultured cloned embryos – its influence on both remodeling of somatic cell-derived chromatin and reprogramming of transcriptional activity of somatic cell genome

Transcriptional activity of somatic cell-inherited nuclear genome during embryo pre- and/or postimplantation development as well as foetogenesis is correlated with the frequencies for spatial remodeling of chromatin architecture and reprogramming of cellular epigenetic memory. These former and latter processes include such covalent modifications as demethylation/*de novo* methylation of DNA cytosine residues and acetylation/deacetylation as well as demethylation/re-methylation of lysine residues of nucleosomal core-derived histones H3 and H4 (Wee et al. 2007, Ding et al. 2008, Lee et al. 2010, Song et al. 2014, Liang et al. 2015, Jin et al. 2017a). In addition, intergenomic communication between heteroplasmically transmitted nuclear DNA, maternally (ooplasmically) inherited copies of mitochondrial DNA (mtDNA) and nuclear donor cell-descended copies of mtDNA affects the profile of gene expression. It also affects the nuclear-ooplasmic interactions in cloned embryos and foetuses (Shi et al. 2004, Yan et al. 2010, 2011). The level of progression for the processes of epigenetic genome-wide alterations that are mediated by histone-lysine methyltransferases (HMTs), DNA methyltransferases (DNMTs 1o and 3a/3b) and histone deacetylases (HDACs) can be modulated (i.e., reversed) via exogenous inhibitors of these enzymes throughout either *in vitro* culture of nuclear donor somatic cells and/or cloned embryos (Martinez-Diaz et al. 2010, Bo et al. 2011, Ning et al. 2013, Sangalli et al. 2014, Huang et al. 2016) or *in vitro* maturation of

nuclear recipient oocytes (Samiec and Skrzyszowska 2012, Samiec et al. 2016). Moreover, the use of the artificial modifiers of epigenomically-conditioned gene expression, leads to the inhibition of both chromatin condensation and transcriptional silencing the genomic DNA of cultured somatic cells that are applied as a source of donor nuclei for the reconstruction of enucleated oocytes and subsequent generation of cloned embryos (Zhao et al. 2010b, Su et al. 2011, Wang et al. 2011a,b). On the one hand, those epigenetic modifiers represent not only the subclass of highly specific/selective extrinsic HMT inhibitors (HMTi) such as G9A (H3K9) HMTi, the pivotal member of which is diazepin-quinazolin-amine derivative termed BIX-01294 [2-(hexahydro-4-methyl-1*H*-1,4-diazepin-1-yl)-6,7-dimethoxy-*N*-[1-(phenylmethyl)-4-piperidinyl]-4-quinazolinamine or *N*-(1-benzylpiperidin-4-yl)-6,7-dimethoxy-2-(4-methyl-1,4-diazepan-1-yl)quinazolin-4-amine] (Huang et al. 2016, Cao et al. 2017), but also the subclass of ectopic non-specific DNMT inhibitors (DNMTi), whose the most important members are: 1) 5-aza-2'-deoxycytidine (5-aza-dC; decitabine) (Enright et al. 2005, Ding et al. 2008, Ning et al. 2013, Huan et al. 2013, 2014, 2015a,b); 2) zebularine (2-pyrimidone-1-β-*D*-riboside; a nucleoside analog of cytidine) (Diao et al. 2013, Xiong et al. 2013); and 3) *S*-adenosylhomocysteine (SAH) (Jeon et al. 2008). On the other hand, they represent the subclass of ectopic non-selective HDAC inhibitors (HDACi), whose main members are: 1) trichostatin A (TSA; [*R*-(*E,E*)]-7-[4-(dimethylamino)phenyl]-*N*-hydroxy-4,6-dimethyl-7-oxo-2,4-heptadienamide) (Li et al. 2008, Cervera et al. 2009, Bo et al. 2011, Saini et al. 2014, Huan et al. 2014, 2015a,b, Samiec et al. 2015, Opiela et al. 2017); 2) valproic acid/2-propylpentanoic acid (VPA) or sodium valproate/sodium 2-propylpentanoate (SV) (Costa-Borges et al. 2010, Kim et al. 2011, Mallol et al. 2014, Sangalli et al. 2014); 3) scriptaid (SCPT; 6-(1,3-dioxo-1*H*,3*H*-benzo[de]isoquinolin-2-yl)-hexanoic acid hydroxyamide) (Van Thuan et al. 2009, Zhao et al. 2009, Xu et al. 2013, Wen et al. 2014, Liang et al. 2015, Samiec et al. 2016); 4) oxamflatin [(2*E*)-5-[3-(phenylsulfonylamino)phenyl]-pent-2-en-4-ynohydroxamic acid or *N*-hydroxy-5-[3-[(phenylsulfonyl)amino]phenyl]-2*E*-penten-4-ynamide or (*E*)-5-[3-(benzenesulfonamido)phenyl]-*N*-hydroxypent-2-en-4-ynamide] (Su et al. 2011, Park et al. 2012, Hou et al. 2014, Mao et al. 2015); 5) sodium butyrate (NaBu) (Das et al. 2010, Liu et al. 2012, Kumar et al. 2013); 6) *m*-carboxycinnamic acid *bis*hydroxamide (CBHA) (Dai et al. 2010, Song et al. 2014); 7) panobinostat, also known as LBH589 [(*E*)-*N*-hydroxy-3-[4-[[2-(2-methyl-1*H*-indol-3-yl)ethylamino]methyl]phenyl]prop-2-enamide] (Jin et al. 2013); 8) abexinostat, also termed PCI-24781 [3-[(dimethylamino)methyl]-*N*-{2-[4-(hydroxycarbamoyl)phenoxy]ethyl}-1-benzofuran-2-carboxamide] (Jin et al. 2016); 9) quisinostat, also called JNJ-26481585 [*N*-hydroxy-2-[4-[[[(1-methyl-1*H*-indol-3-yl)methyl]amino]methyl]-1-piperidinyl]-5-pyrimidinecarboxamide] (Jin et al. 2017a); 10) dacinostat, also named as LAQ824 or NVP-LAQ824 [(E)-*N*-hydroxy-3-[4-[[2-hydroxyethyl-[2-(1*H*-indol-3-yl)ethyl]amino]methyl]phenyl]prop-2-enamide] (Jin et al. 2017b); 11) belinostat, also known as PXD101 [(*E*)-*N*-hydroxy-3-[3-(phenylsulfamoyl) phenyl]prop-2-enamide or *N*-hydroxy-3-[3-(phenylsulfamoyl) phenyl]-2-propenamide] (Qiu et al. 2017); and 12) bromotyrosine-derived, symmetrical conjugate of cystamine (antibiotic first isolated from the *Psammaplinaplysilla* marine sponge and probably exhibiting also the DNMTi and anti-tumor activity), designated as psammaplin A or bisprasin, (PsA; *N*,*N''*-(dithiodi-2,1-ethanediyl)bis[3-bromo-4-hydroxy-a-(hydroxyimino)-benzenepropanamide or (2*E*)-3-(3-bromo-4-hydroxyphenyl)-*N*-[2-[2-[[(2*E*)-3-(3-bromo-4-hydroxyphenyl)-2-hydroxyiminopropanoyl]amino]ethyldisulfanyl]ethyl]-2-hydroxyiminopropanamide) (Mallol et al. 2014, 2015, 2016). The onset of chromatin decondensation and gene transcriptional activity is evoked both via highly specific/selective and transient inactivation of G9A (H3K9) HMTs by BIX-01294 (Huang et al. 2016, Cao et al. 2017) and via non-specific/non-selective (i.e., broad-spectrum) blocking the biocatalytic activity of either DNMTs by 5-aza-dC, zebularine and SAH (Jeon et al. 2008, Diao et al. 2013, Huan et al. 2015a,b) or HDACs by TSA, VPA/SV, SCPT, oxamflatin, NaBu, CBHA, panobinostat, abexinostat, quisinostat, dacinostat, belinostat and PsA (Kim et al. 2011, Park et al. 2012, Jin et al. 2013, Kumar et al. 2013, Xu et al. 2013, Song et al. 2014, Mallol et al. 2015, Jin et al. 2016, 2017a,b, Qiu et al. 2017). Such exogenous epigenomic modulation (epigenetic transformation) of nuclear donor cells, nuclear recipient cells and/or cloned embryos may facilitate and accelerate the reprogrammability for gene expression of donor cell nuclei that have been transplanted into cytoplasmic microenvironment of recipient oocytes and subsequently undergo the de-differentiating and re-establishing the epigenetically dependent status of their transcriptional activity during the preimplantation development of cloned embryos (Van Thuan et al. 2009, Martinez-Diaz et al. 2010, Bo et al. 2011, Huan et al. 2015a,b, Huang et al. 2016, Jin et al. 2017b). The indirect exogenous DNMTi- and/or direct ectopic HDACi-induced hyperacetylation of lysine residues on nucleosomal core-related histones H3 and H4 can play a role of epigenetic recognition coding system for the increased recruitment of histone acetyltransferases (HATs) and

the enhanced association of double bromodomain-containing chromatin adaptor protein-4 (BRD4) molecules to acetylated histones of meiotic condensed chromosomes in the *in vitro*-maturing oocytes. It has been shown that the preferential binding of BRD4 proteins to lysine moieties of hyperacetylated core histones forming the octomeric nucleosome contributes to the rhythmic conversion of transcriptionally repressive chromatin (heterochromatin) to the transcriptionally permissive chromatin (euchromatin) (Rybouchkin et al. 2006, Nagashima et al. 2007, Wang et al. 2011b, Liang et al. 2015, Gonzales-Cope et al. 2016, Cao et al. 2017). As a result, after replacement of metaphase II chromosomes in the oocytes with the somatic cell-inherited chromatin, extrinsic (e.g., TSA- or SCPT-mediated) inhibition of global histone deacetylation through down-regulation of HDAC activity can facilitate and accelerate the architectural remodeling and epigenetic reprogramming processes of nuclear donor cell-descended chromatin within the preimplantation cloned embryos. It is, therefore, conceivable that the erasing of epigenomic memory can occur in the somatic cell nuclei after their introduction into the cytoplasm of enucleated oocytes (Das et al. 2010, Zhao et al. 2010b, Samiec et al. 2016, Opiela et al. 2017, Qiu et al. 2017). This can thereby give rise to the remarkable transformation of cytosine residue methylation marking of nuclear donor DNA from epigenetic pattern of differentiated cells into the totipotent dedifferentiated status of embryonic (i.e., zygotic) cells. Furthermore, the establishment of a transcriptionally permissive chromatin state within the rearranged donor cell nuclei via inducible active acetylation of histones H3 and H4 followed by indirect genome-wide demethylation of DNA cytosine residues can cause the silencing of gene expression to cease in the cells of nuclear-transferred (NT) embryos developing to the morula and blastocyst stages (Wee et al. 2007, Shi and Wu 2009, Wang et al. 2011a, Diao et al. 2013, Hou et al. 2014, Samiec et al. 2015, Jin et al. 2016, 2017a).

Conclusions and future goals

Incomplete and aberrant reprogramming of epigenetic memory of somatic cell nuclei in preimplanted nuclear-transferred (NT) embryos is one of the most important factors that limit the cloning effectiveness (Bonk et al. 2008, Buganim et al. 2013, Nashun et al. 2015). The extent of epigenetic genome-wide alterations involving DNA methylation and histone deacetylation that are mediated by DNA methyltransferases (DNMTs) and histone deacetylases (HDACs) can be modulated/reversed via exogenous inhibitors of these enzymes throughout *in vitro* culture of nuclear donor cells, nuclear recipient oocytes and/or cloned embryos (Su et al. 2011, Wang et al. 2011a, Mason et al. 2012, Gonzales-Cope et al. 2016). The use of the artificial modifiers of epigenomically-conditioned gene expression leads to inhibition of both chromatin condensation and transcriptional silencing the genomic DNA of somatic cells that provide a source of nuclear donors for reconstruction of enucleated oocytes and generation of cloned embryos (Martinez-Diaz et al. 2010, Fisher and Fisher 2011, Song et al. 2014). The onset of chromatin decondensation and gene transcriptional activity is evoked via highly specific blocking the activity of HMTs by BIX-01294 (Huang et al. 2016) and via broad-spectrum blocking the activity of either DNMTs by 5-aza-2'-deoxycytidine, zebularine, *S*-adenosylhomocysteine (Jeon et al. 2008, Huan et al. 2013, Xiong et al. 2013) or HDACs by trichostatin A, valproic acid/sodium valproate, scriptaid, oxamflatin, sodium butyrate, *m*-carboxycinnamic acid *bis*hydroxamide, panobinostat, abexinostat, quisinostat, dacinostat, belinostat and psammaplin A (Dai et al. 2010, Bo et al. 2011, Kim et al. 2011, Wang et al. 2011b, Jin et al. 2013, Ning et al. 2013, Mallol et al. 2014, Mao et al. 2015, Samiec et al. 2015, Jin et al. 2016, 2017a,b, Qiu et al. 2017). Epigenomic modulation of nuclear donor cells, nuclear recipient cells and/or cloned embryos may facilitate and accelerate the reprogrammability for gene expression of donor cell nuclei that have been transplanted into a host ooplasm and subsequently underwent dedifferentiating and re-establishing the epigenetically dependent status of their transcriptional activity during pre- and postimplantation development of NT embryos (Eilertsen et al. 2007, Shi and Wu 2009, Costa-Borges et al. 2010, Samiec and Skrzyszowska 2012, Hou et al. 2014, Samiec et al. 2016, Cao et al. 2017, Opiela et al. 2017).

Summing up, while tremendous progress in the field of somatic cell cloning has been achieved during the past few years with the birth of numerous offspring of different mammalian species worldwide, the overall efficiency remains low. The current high incidence of pre- and/or postimplantation embryonic, foetal as well as perinatal abnormalities limits the practical applications of somatic cell cloning and contributes to the negative perception of this assisted reproductive technology (ART) to society. The aims are to understand the mechanisms involved in the aberrations for both wide epigenetic transcriptional reprogramming of donor cell-descended nuclear DNA and differential (maternal or paternal) expression patterns of several imprinted (i.e., uniparentally-expressed) genes that can lead to the pathologic syndromes in cloned foetuses and neonates (Zhao et al. 2010a, Des-

hmukh et al. 2011, Esteves et al. 2011, Anckaert and Fair 2015, Jafarpour et al. 2017, Masala et al. 2017).

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