—Mini Review—

Epigenetic Treatment in Assisted Human Embryo Implantation

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Abstract: *Recent advances in assisted reproductive technologies (ART) have resulted in higher fertilization rates for infertile couples. However, successful pregnancy is prevented by failure of the embryo to implant in the endometrium. Human embryo implantation involves apposition and adhesion, followed by penetration through the endometrial epithelial layer and invasion of the embryo into the endometrial stroma. Human implantation is a multi-step event requiring the orchestrated regulation of endometrial cells, involving cell proliferation, differentiation, motility, and adhesion. Reversible histone acetylation affects functional protein expression by regulating gene transcription. Histone deacetylase inhibitors (HDACIs) can induce expression of specific proteins, thereby affecting cell function. At present, HDACIs are used in anticancer therapy to induce cancer cell apoptosis. In this review, we discuss the potential of HDACIs for supportive therapy of infertility caused by endometrial dysfunction, and introduce recent reports that HDACIs target and affect various cell functions.*

Key words: *HDACI, Glycodelin, Implantation*

Introduction

In spite of recent high fertilization rates because of advanced *in vitro* fertilization technology, the pregnancy rate in assisted reproduction has plateaued because of implantation failures. Ethical concerns limit *in vivo* studies of human embryo implantation within the "black box" uterine cavity. Human implantation is a multi-step process, involving embryo apposition/adhesion, penetration through the endometrial epithelial layer, and invasion into the endometrial stroma. Coordinated regulation of endometrial cell functions that involve cell proliferation, differentiation, movement, and adhesion is necessary for successful implantation. Progesterone can regulate these multistep alterations of endometrial cells, and for endometrial dysfunction or clients undergoing *in vitro* implantation and embryo transfer, additional progesterone therapy (luteal support) has been performed; however, it is not obvious that improved results have been obtained. It is disappointing that the present strategy using hormonal support for assistance of implantation has not been further developed. Because of the shortcomings in the current state of ART for assisted implantation, there is now interest in the development of a novel reagent that will induce orchestrated endometrial regulation of human implantation.

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Histone Deacetylase Inhibitors

To condense and store long genome sequences within the chromosome, 146 nucleotide base pairs are wrapped around a histone protein core in each 1.75 turns of the DNA superhelix [1]. The acetylation status of core histones is reversibly and cooperatively regulated by two groups of enzymes, histone acetyltransferases (HAT) and histone deacetylases (HDAC) (Fig. 1) [2]. In the deacetylated steady state, gene silencing is structurally accompanied by the tightly arranged nucleosome structure. In contrast, when histones are acetylated, neutralization of the positive charge results in a loosened nucleosome arrangement that makes promoter regions readily accessible to transcription factors (Fig. 1). Through association of transcription factors with promoter regions, followed by

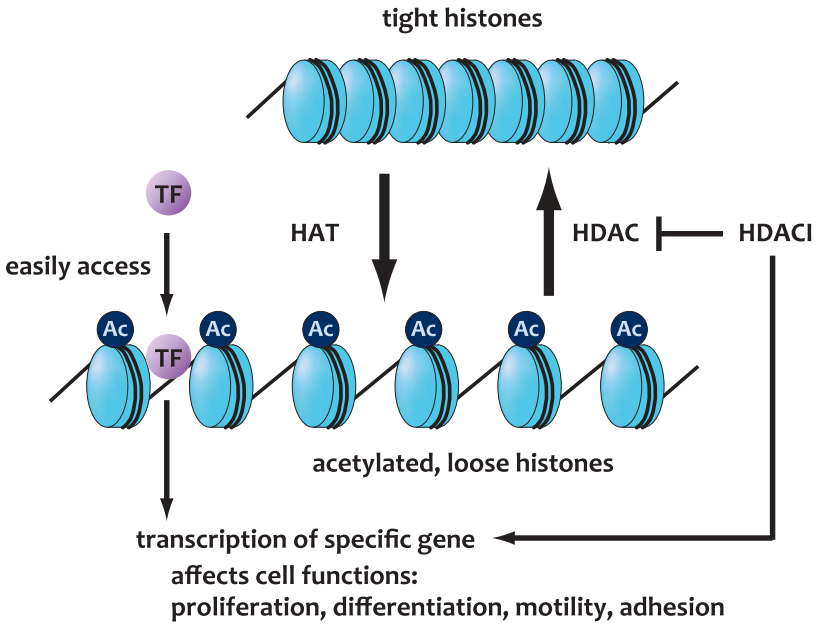

Fig. 1. Reversible histone acetylation and gene transcription. The histone proteins wrapped in the human genome are reversibly acetylated by histone acetyltransferases (HAT) and histone deacetylases (HDAC). Addition of a histone deacetylase inhibitor (HDACI) results in hyperacetylation of histones, and thereby causes alterations in specific gene transcription by allowing easy access to transcription factor (TF).

defined gene transcription, multiple cellular functions that depend on the transcribed gene, such as cell proliferation or apoptosis, cell differentiation, and cell motility are affected. Taking into consideration these physiological mechanism(s), it is easy to predict that artificial alteration of histones to a hyperacetylated state by a histone deacetylase inhibitor (HDACI) may alter regulation of the cell functions that are important for cell survival and development.

HDACIs & Cell Differentiation

During the first steps of human implantation, the embryo apposes and then adheres to the surface layer of the endometrial epithelial barrier. Similar to the rolling leukocyte phenomenon in which the leukocyte apposes and adheres to the damaged endothelial cell lining of a blood vessel [3], the floating fertilized egg and receptive endometrial epithelial cells require specific mediators such as chemokines and adhesion molecules to prepare for acceptance of each other [4, 5]. Changes in cell polarity for the benefit of adhesion-related molecular rearrangement also occur during implantation [6, 7]. Cytodifferentiation can result from the appropriate regulation of adhesion-related molecules and cell polarity. We have previously reported that an HDACI, trichostatin A (TSA), can induce the production of a protein marker of endometrial stromal cell differentiation, the insulin-like growth factor binding protein-1 (IGFBP-1), and prolactin in human endometrial stromal cells, thereby potentiating decidualization [8]. Although stromal cells are one of the major components of the human endometrium, luminal and glandular epithelial cells, other major components are also important because they are located in the front line of the endometrium and therefore have the unique potential to directly interact with the embryo. We demonstrated that similar to stromal cells, the human endometrial epithelial cell line, Ishikawa, derived from adenocarcinoma, can be induced by suberoylanilide hydroxamic acid (SAHA) and TSA to produce glycodelin and leukemia inhibitory factor (LIF), which are marker proteins of endometrial epithelial differentiation, at both the levels of mRNA and protein expression [9].

Glycodelin is a 28 kDa secretory protein that has a unique temporo-spacial expression pattern. It is primarily expressed in reproductive organs such as

uterine endometrium, cervical glands, Fallopian tubes, ovaries, and mammary glands [10]. In the human breast cancer cell line, MCF-7, SAHA induces milk-fat globule protein, milk fat membrane-globule protein, and lipid droplets [11]. Although glycodelin can be induced by the addition of SAHA to MCF-7 cells [12], interestingly, transfection of glycodelin cDNA into MCF-7 breast cancer cells induced a dramatically altered growth behavior, with formation of acinar configurations, and an inability to grow in semisolid media because of apoptosis [13].

In human endometrial glands, glycodelin is minimally expressed during the estradiol (E2)-based proliferative phase. Its expression is induced by progesterone exposure in the early secretory phase, peaks 10 days after ovulation, and persists until menstruation begins or pregnancy is established. The period of glycodelin expression has been referred to as the "implantation window" [10]. Glycodelin expression can be induced by treatment with ovarian steroid hormones (E2 combined with progesterone [P4]) or an HDACI (TSA or SAHA) through activation of Sp1 in the glycodelin promoter region [9]. Addition of E2P4 or SAHA can also induce spreading- and flattened-cell morphological changes and glycogen synthesis, which are blocked by glycodelin small interfering RNA (siRNA) transfection [9]. All of these results indicate that HDACI-inducible glycodelin can affect endometrial epithelial cell differentiation, thereby providing appropriate conditions for accepting the embryo.

HDACIs & Implantation (Adhesion)

After apposition, the fertilized egg adheres and spreads onto the endometrial epithelium and then penetrates the epithelium (Fig. 2). It has been reported that several membrane proteins in endometrial epithelial cells are required for feto-maternal adhesion, including L-selectin [4, 5], and E-cadherin [14, 15]. Takai *et al.* have shown that SAHA induces E-cadherin expression in Ishikawa cells [16]. As mentioned previously, the peak expression of glycodelin occurs in the mid-secretory phase (implantation window) [10]. In an *in vitro* implantation assay, E2P4- or SAHA-treated Ishikawa cells developed increased adhesiveness to an *in vitro* embryo model (spheroid of the JAR choriocarcinoma cell line) [12]. Adhesion to an embryo model is cell-type dependent, NIH3T3 cells (fibroblast) and HeLa cells (human cervical cancer cell line) showed no obvious adhesiveness to the embryo model. In addition the cell line MCF-7, which can be induced to moderately express glycodelin, adheres at a moderate level to JAR spheroids. Furthermore, Ishikawa cell adhesion to the embryo model is almost completely abolished by siRNA glycodelin gene silencing [12]. Interestingly, the enhancing effect caused by E2P4 or SAHA treatment in the embryo model can be observed not only in the adhesion ratio between Ishikawa cells and JAR spheroids, but also in the area of spreading (HU, unpublished data). Taken together, SAHA-induced glycodelin can aid in embryo adhesion to the endometrial epithelial cell layer, and probably affects embryo spreading (penetration horizontally).

HDACIs & Cell Proliferation

Robust endometrial epithelial cell proliferation may cause difficulty in providing space for embryo penetration. It is compatible with the concept that cell apoptosis occurs even in mouse embryo implantation [17]. At present an HDACI is used clinically as a cancer therapeutic, because it can induce cancer cell apoptosis. SAHA (Zolinza®) has been approved by the U.S. Food and Drug Administration (FDA) for the treatment of some types of lymphoma. In MCF-7 cells SAHA inhibits cell proliferation, and in a dose-dependent manner, accumulation of cells in the G1 and G2-M phases of the cell cycle [11]. In an endometrial epithelial cell line (Ishikawa), treatment with SAHA significantly decreased cell proliferation, and decreased the proportion of cells in the S phase and increased the G0-G1 and/or G2-M phase cell proportions through the down-regulation of cyclin D1, cyclin D2, Bcl-2, and p21 proteins [16]. As mentioned previously, we also demonstrated that SAHA treatment induced glycodelin protein expression [9]. Although somewhat contradictory to the results for SAHA-treated Ishikawa cells, we have also reported that glycodelin overexpression by cDNA transfection inhibited cell growth through up-regulation of p16, p21, and p27 mRNA expression, as well as increasing the proportion of cells in the G1 phase and decreasing the S and G2/M phases proportions [18]. These results indicate that SAHA treatment and its induced glycodelin can suppress endometrial epithelial cell proliferation and that suppression may be beneficial to counteract resistance to embryo penetration (Fig. 2).

HDAIs & Cell Motility

How is the endometrial epithelial cell sheet barrier that is penetrated by the embryo repaired? In textbook

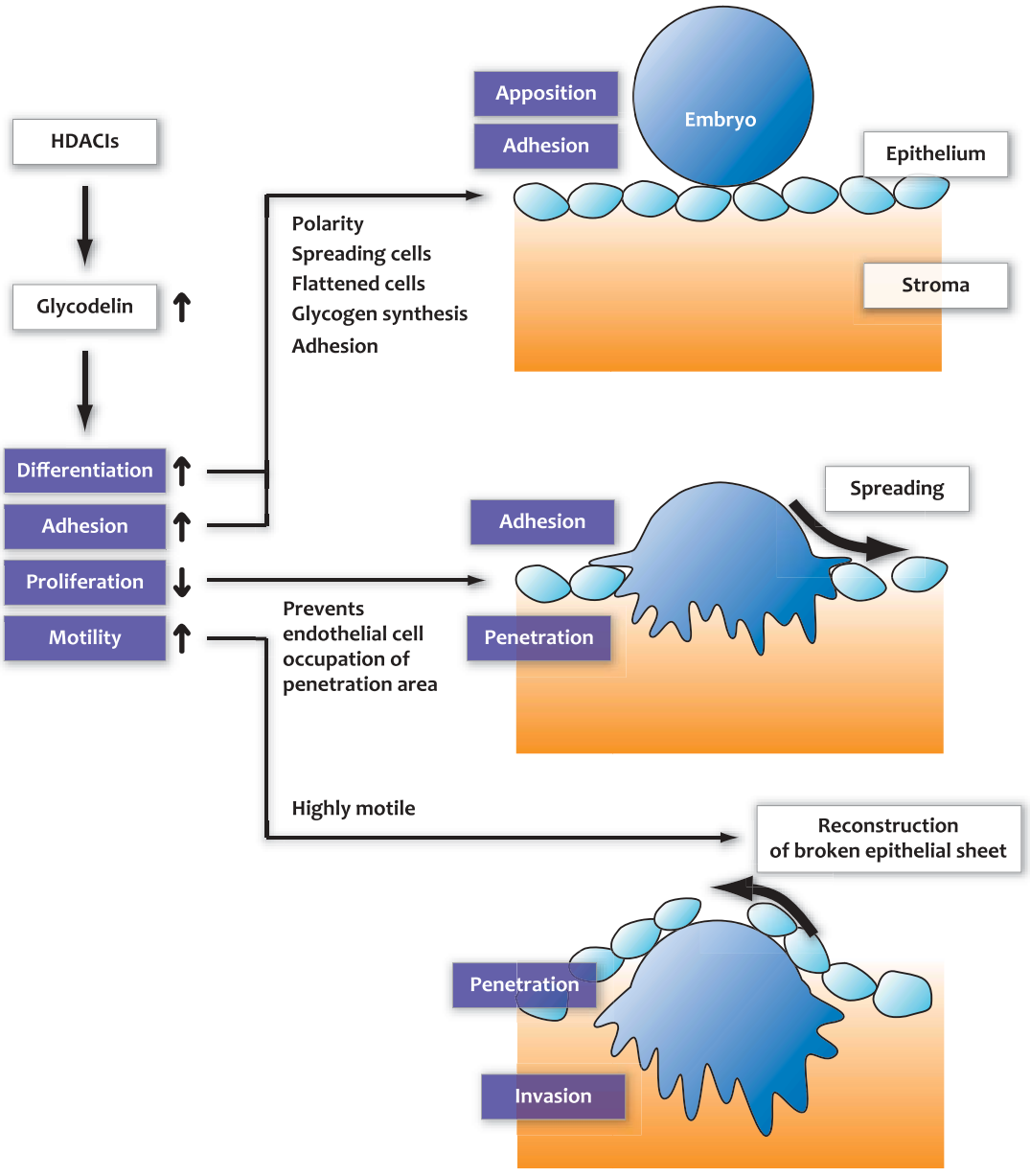

Fig. 2. Multistep process of human implantation is affected by HDACI through up-regulation of glycodelin. Treatment with HDACI induces up-regulation of glycodelin expression in human endometrial epithelial cells, and thereby causes enhancement of cell differentiation, induction of cell-cycle arrest, increased adhesion to the embryo, and cell motility acceleration. All these phenomena have advantages for every step of human implantation.

figures of human implantation, fibrin formation is traditionally drawn at the edge of the gap surrounding the remaining epithelium; however, the repair mechanism of the damaged epithelial sheet is not specifically described. To avoid implantation failure (i.e., detachment of the adhered and penetrating embryo), the damaged epithelial layer must be quickly reconstructed. This means that the gap caused by embryo penetration should be repaired by rapid cell migration, rather than by slow cell proliferation (Fig. 2). Of course, to occupy space, changes in cell morphology, cell spreading and flattening, are also necessary, in addition to cell migration. In the E2P4- or SAHA-treated Ishikawa cells, cell motility is highly

accelerated in both single cell (transmigration assay) and in collective cells (wound healing assay) [19]. These phenomena, which are induced by elevated glycodelin expression, are blocked by glycodelin siRNA transfection [19]. The report that glycodelin peptide transfection into human umbilical vein endothelial cells (HUVEC) caused up-regulation of cell migration activity, supports the theory that up-regulation of endometrial epithelial cell motility is due to glycodelin [20].

In the 1990s, glycodelin was thought of as an immunosuppressive protein, because of its inhibitory effect on natural killer cells [21]. This activity indicates that glycodelin permits semi-allograft (embryo) transplantation. The addition of purified glycodelin protein blocks migration in the human monocyte cell line, U937 [22]. Glycodelin induced by E2P4 or HDACI, accelerates endometrial epithelial cell migration and decelerates immune cell migration. These activities, concerned with reconstructing the damaged epithelial cell sheet barrier and providing immunosuppression at the implantation site, make glycodelin suitable for use in assisting human embryo implantation.

HDACIs & Embryo

Although SAHA (and its induced protein, glycodelin) appears to provide assistance for the multistep process of human implantation, their effects on cell differentiation, adhesion, proliferation, and motility, fetal and maternal safety must be strictly validated. In healthy humans it is well known that SAHA administration has no severe side effects from clinical experience using it as an anti-lymphoma agent; however, its effects on the human embryo have not been investigated. Although, surprisingly, only up to 20% of the genome is controlled by HDAC, the HDACI valproic acid (VPA) has caused “valproate syndrome”. Valproate syndrome has been described in babies from VPA-treated mothers and is characterized by spina bifida, craniofacial anomalies, and a range of less frequent malformations. In a mouse study, both TSA and VPA induced hyperacetylation in embryos one hour after treatment. Fusions of vertebrae and ribs, duplications of segments, and homeotic transformation of segments with no external, craniofacial, or limb abnormalities, were recorded at term [23]. In an *in vitro* implantation assay we used SAHA for endometrial epithelial cell stimulation, prior to co-culture with the embryo model [12], and observed that SAHA effects on cells were transient and reversible. Consequently, as in our experimental model, an embryo safety validation study should include testing SAHA on endometrial epithelial cells prior to co-culture with the embryo.

Conclusion

A variety of human endometrial epithelial cell functions are affected by HDACIs, including differentiation, adhesion, proliferation, and motility. Because these effects are advantageous for human implantation, HDACI has potential as an epigenetic treatment in assisted reproduction treatments for infertile women.

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