Novel Insights into the Control of Human Pregnancy: Potential Role(s) for Epigenetic Regulation

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Abstract: The appropriate modulation of gene expression in gestational tissues is an essential requirement for a successful pregnancy. Epigenetic control of gene expression through the reversible modification of chromatin has emerged as a fundamental mechanism for the coordination of gene expression in a range of biological systems. Here we summarize recent results in support of our hypothesis that key physiological events and pathological processes occurring throughout human pregnancy are under epigenetic control.

Keywords: epigenetics, pregnancy, gene expression, DNA methylation, histone modification

Introduction

Human pregnancy comprises a complex series of differentiation and growth processes that must be precisely coordinated in time and space if a successful outcome is to result. The appropriate, concerted modulation of gene expression across the range of cell types found in gestational tissues is thus a fundamental requirement for a successful pregnancy.

Epigenetic control of transcription through the reversible modification of chromatin has emerged as a cardinal mechanism for the coordination of gene expression in a range of biological systems (Bernstein et al. 2007). The best-studied forms of chromatin modification are DNA methylation and histone acetylation; it appears that these processes are interactive (Fuks, 2005), such that transcriptional silencing is associated with concurrent DNA methylation and histone deacetylation (Fig. 1). Both classes of modification can be reversed through treatment with a chemical inhibitor: 2'-deoxy-5-azacytidine (AZA) inhibits DNA methylation leading to global hypomethylation of DNA, while treatment with an inhibitor of histone deacetylation such as trichostatin (TSA) leads to increased levels of histone acetylation. In both cases, intervention favours the transcriptionally active epigenetic state.

Epigenetic mechanisms are known to underlie the phenomenon of genomic imprinting, in which certain genes are expressed in a parent-specific manner (Duranthon et al. 2008; Rivera et al. 2008; Watkins et al. 2008), and have been increasingly associated with long-term changes to gene expression programs in response to changes in the gestational environment (Gluckman and Hanson, 2004). Recent results from our laboratory and from others suggest that epigenetic mechanisms are also centrally involved in the regulation of key physiologic processes in human pregnancy. We have recently advanced an epigenetic model of parturition (Mitchell, 2006; Sato and Mitchell, 2006) in which the transition to labor is coordinated by a change in epigenetic status within gestational tissues. Here we survey recent reports in support of this hypothesis, and of our further suggestion that epigenetic processes constitute the cardinal regulatory basis for the coordination of gene expression in maternal and placental tissues throughout pregnancy. We suggest that future research will reinforce the importance of epigenetic status as a key indicator of pathological pregnancy, providing the basis for new methods of diagnosis and perhaps for novel interventions targeting aberrant epigenetic states.

Maintenance of Pregnancy and the Initiation of Labor—Evidence for Epigenetic Regulation

Pregnancy is maintained through persistent damping of uterine responsiveness to inflammatory or contractile stimuli; conversely, labor is the result of transition of the myometrium to an active state of contraction.

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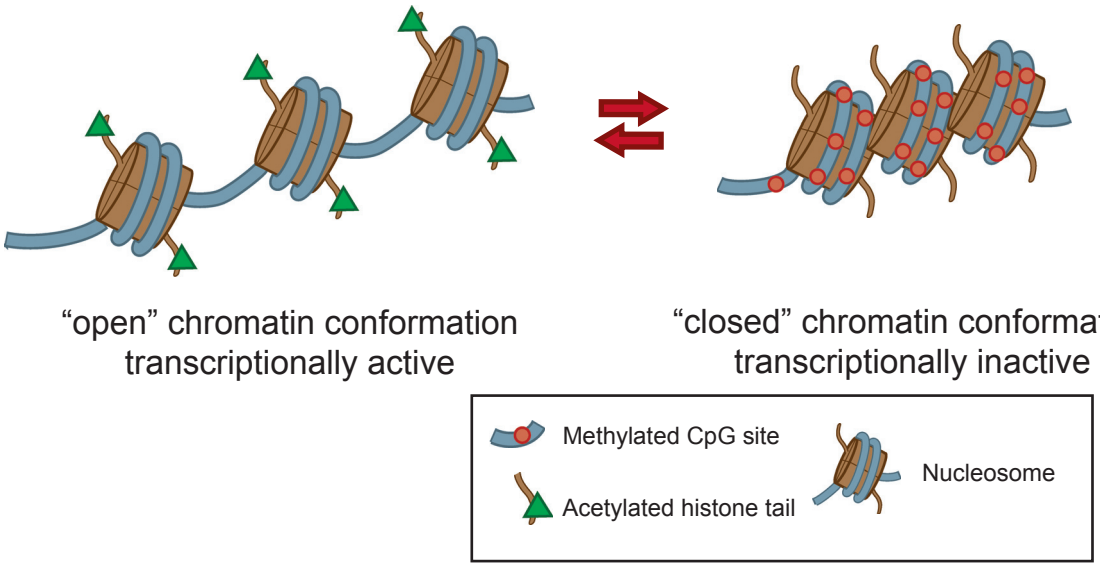

Figure 1. Epigenetic regulation of transcription. DNA methylation and histone deacetylation are epigenetic marks associated with a condensed chromatin conformation and repression of transcription.

The molecular events that underlie this transition are not well understood, however functional progesterone withdrawal is presumed to be involved. In humans, neither plasma progesterone levels nor uterine progesterone receptor (PR) levels decrease near term (Roh et al. 1999; Challis et al. 2000), yet administration of the progesterone receptor (PR) antagonist RU486 increases uterine contractility (Herrmann et al. 1982), indicating that functional progesterone withdrawal at term is mediated by molecular processes that modulate the transcriptional activity of the progesterone—PR complex. Condon and Mendelson have described alterations in the ratio of PR isoforms in human myometrium near term that are consistent with a lowered responsiveness to progesterone (Condon et al. 2006), and have also investigated the potential role of changes in the levels of co-regulators essential to the transcriptional activity of the progesterone—PR complex in mediating functional progesterone withdrawal (Condon et al. 2003). A significant down-regulation in the expressions of steroid receptor coactivators (SRCs) 2 and 3 in human myometrium after the initiation of spontaneous labor was observed; importantly, SRC-3 contains histone acetyltransferase activity, and the observed decline in co-activator expression was associated with a marked decrease in histone H3 acetylation in the myometrium, which might result in a remodelling of chromatin structure causing a decrease in transcriptional activity of the progesterone—PR complex. Consistent with these findings, administration of TSA to mice during late gestation led to hyperacetylation of uterine histone H3, and delayed the initiation of spontaneous labor by up to 48 hrs. These results suggest that uterine epigenetic status constitutes a key component of the molecular pathway regulating functional progesterone withdrawal, and hence the initiation of labor.

In addition to functional progesterone withdrawal, an increase in intrauterine prostaglandin (PG) production is known to be a key step in the transition to labour (Keelan et al. 2003). The activity of prostaglandin H synthase-2 (PGHS-2, a key PG biosynthetic enzyme) has been shown to be suppressed by enhanced DNA methylation and reduced histone acetylation in specific cancer tissues (Song et al. 2001; Suzuki et al. 2002; Toyota et al. 2000); we hypothesised that the transition to labour might similarly be characterised by changes in the epigenetic status of key prostaglandin biosynthesis and metabolism genes to favour intrauterine prostaglandin production. Thus we treated human term amnion explants with inhibitors of DNA methylation and histone deacetylation, and determined the effects of these treatments both on basal and stimulated PG production, and on the expressions of key enzymes of PG biosynthesis and metabolism. We found that whereas treatment with IL-1β resulted in large increases in PGE_2 production in term amnion, this response was reduced through co-treatment with an inhibitior of DNA methylation, and completely abrogated through concurrent inhibition of histone deacetylation

(Mitchell, 2006). Further studies revealed that these effects were mediated through changes in the responsiveness of PGHS-2 and PGDH expression to IL-1β stimulation, such that both the induction of PGHS-2 and the relative down-regulation of PGDH normally caused by IL-1β stimulus were attenuated. We speculate that these changes constitute reversion to an epigenetic state associated with uterine quiescence, in which the overall responsiveness of amnion to an inflammatory stimulus is lowered. We believe that in combination with the epigenetic mechanism for functional progesterone withdrawal described by Condon and Mendelson, this mechanism for the modulation of uterine responsiveness to contractile stimulus constitutes an epigenetic model for the overall coordination of the initiation of labor (Fig. 2).

Increased production of inflammatory cytokines in response to ascending intrauterine infection, with subsequent increased intrauterine PG production, is recognised to be a major causative factor in the preterm initiation of labor leading to premature birth (Keelan et al. 2003; Romero et al. 2005; Romero et al. 2006). Through reducing the responsiveness to inflammatory stimulus of amnion, the key site of intrauterine PG production, the epigenetic switch described above presumably functions to oppose premature initiation of labor. Somewhat surprisingly therefore, we found that co-treatment with an inhibitor of histone deacetylation massively *increased* the stimulatory effect of the bacterial endotoxin lipopolysaccharide (LPS) on IL-1β production in term choriodecidual explants (Sato and Mitchell, 2006).This area clearly requires further investigation; it is noteworthy that the concentration dependence of the response was not evaluated, and this could affect interpretation of the result. However the fact that corresponding responses were not observed with respect to TNF-α, IL-10, or IL-1Ra production leads us to speculate that our results implicate a new regulatory pathway that is specific to the production of IL-1β. It has been shown that IL-1β plays a critical role in early pregnancy (Feinberg et al. 1994; Ross et al. 2003); we suspect that the same epigenetic state that enhances stimulated IL-1β production in choriodecidual tissue concomitantly reduces the responsiveness of the adjacent amnion and myometrium, thereby allowing necessary surges in local production to occur without the inadvertent initiation of premature labor.

Increased activity of matrix-degrading enzymes within the fetal membranes is thought to contribute to preterm premature rupture of membranes (PPROM), the leading identifiable cause of preterm birth (Vadillo-Ortega and Estrada-Gutierrez, 2005). Wang et al. have recently reported (Wang et al. 2008) that inhibition of DNA methylation in human amnion fibroblasts resulted in significantly increased expression of matrix metalloprotease 1 (MMP1; a key enzyme involved in extracellular matrix turnover), which was correlated with reduced DNA methylation at a particular site in the MMP1 promoter. A new T > C single nucleotide polymorphism in the MMP1 promoter was also identified; the minor C allele was always methylated in vivo, exhibited reduced promoter activity, and in a case–control study was found to be protective against PPROM. The authors concluded that the expression of MMP1, and hence one risk

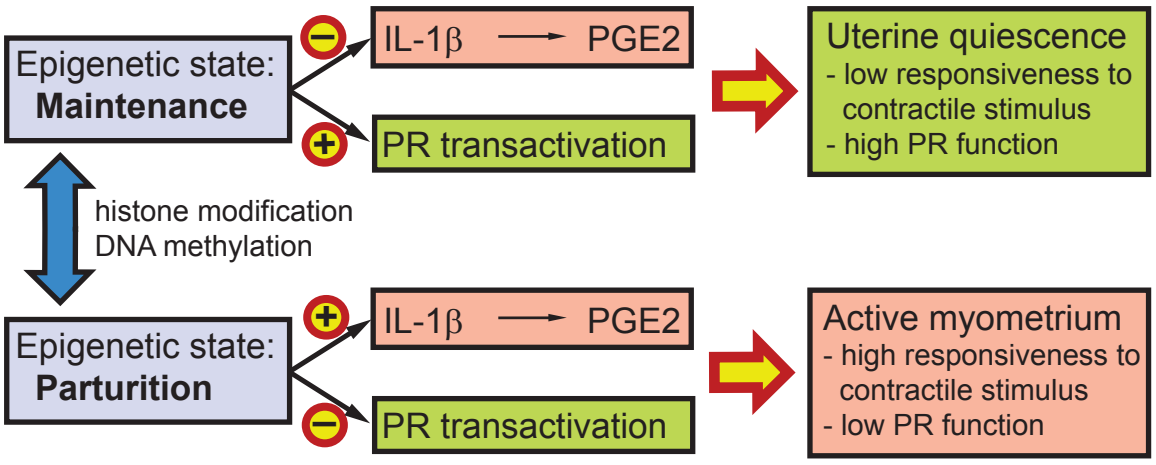

Figure 2. An epigenetic model for maintenance of pregnancy and the initiation of labour: both uterine responsiveness to contractile stimulus and PR transcriptional activity are coordinated by epigenetic states signalling "maintenance" and "parturition" status.

factor for PPROM, is controlled by a combination of epigenetic and genetic mechanisms.

Epigenetic Regulation of Early Pregnancy

The results discussed above are strongly supportive of our suggestion that epigenetic processes play a central role in the regulation of parturition. Recently we have broadened our investigations to include the early stages of pregnancy, which involve dramatic and concerted changes in cell morphologies and expression profiles (Norwitz et al. 2001). While this work is still at an early stage our initial findings are intriguing, and support our contention that the proposed epigenetic model of parturition can be extended to cover all the major phases and events of pregnancy.

Trophoblast invasion

Placentation involves a complex and precisely-regulated process of growth and differentiation in cells of the trophoblast lineage, leading to invasion of the endometrium and re-modelling of maternal spiral arteries by "pseudo-malignant" extra-villous trophoblasts. The transition of epithelial cytotrophoblasts to an invasive mesenchymal form represents a striking change in phenotype that mirrors the epithelial-mesenchymal transition seen in malignant carcinomas (Ferretti et al. 2007). Given that the latter process has been associated with changes in DNA methylation status in key tumour suppression genes, we sought to investigate the impact of altering methylation status on invasive potential using in vitro models of trophoblast invasion (Rahnama et al. 2006). Thus we treated invasive trophoblast-derived BeWo cells with an inhibitor of DNA methylation, and determined the effects on cell morphology and motility. We found that hypomethylation caused BeWos to revert to a non-invasive morphology, with corresponding loss of invasive potential as measured by wound healing and transwell migration assays. Examination of the expression levels of adherens junction components revealed increased expressions of E-cadherin and γ-catenin at the RNA and protein levels in treated cells, suggesting that the observed loss of invasive potential is the result of increased cell-cell adhesion. siRNA-mediated depletion of DNA methyl transferase activity similarly decreased invasive potential, confirming that motility was dependant on DNA methylation status. Although these experiments were performed in choriocarcinoma cells rather than in primary trophoblasts, our results are consistent with the observed association between adherens junction component expression and invasiveness in trophoblasts (Shih Ie et al. 2002), and we expect that future work in primary trophoblast culture will confirm them.

Uterine receptivity

The successful attachment of the blastocyst to the uterine wall is a key process in human pregnancy that marks the beginning of the intimate phase of the fetal-maternal dialogue. Attachment is only possible in that part of the menstrual cycle during which the uterine epithelium enters a receptive state, characterized by a significant change in cell morphology and in the repertoire of cell surface molecules displayed; we recently undertook to investigate whether epigenetic processes underlie these cyclical changes in cell phenotype. Using an in vitro model of uterine receptivity, we have been able to characterise the receptivity of endometrial cell lines toward BeWo spheroids which model the early blastocyst. Our preliminary results indicate that inhibition of DNA methylation results in a significant up-regulation of E-cadherin expression in the non-receptive cell line studied, associated with an increase in receptivity (Thompson et al. 2007).

Decidualisation

The successful transformation of endometrial stromal cells into larger, rounder decidual cells with an enlarged nucleus is a prerequisite of implantation in human pregnancy. The process of decidualisation involves substantial, well-regulated modification of stromal cell expression profiles, and as such is likely to be controlled by epigenetic processes. The impact of modification of epigenetic status on cell phenotype in an endometrial cell line is currently under study in our laboratory; our initial results indicate that hypomethylation does indeed induce morphological and biochemical changes that are consistent with decidualisation (Logan et al. 2007).

Conclusion and Outlook

Successful pregnancy requires the precisely coordinated modulation of gene expression in

gestational tissues, and as such is a process in which epigenetic regulation of gene expression is likely to play a key role. The recent results described here suggest that this is indeed the case, and lead us to speculate that each of the key steps in human pregnancy may be initiated through an epigenetic switching event. We expect that further research will reinforce this view, and will allow the full elucidation of the molecular changes that result from such shifts in epigenetic status.

DNA methylation status is attracting increasing attention as a potential basis for new methods for the early prognosis, and perhaps treatment, of various forms of cancer (Das and Singal, 2004). The clinical use of histone deacetylase inhibitors to target aberrant histone acetylation status in cancers (Bolden et al. 2006; Kelly and Marks, 2005) is similarly under active investigation. We foresee an expansion of current research efforts (Condon et al. 2003; Lindstrom et al. 2008; Phillips et al. 2005) around the development of new clinical approaches to disorders of pregnancy based on the identification and treatment of aberrant epigenetic states. Ultimately, we anticipate that epigenetic status will become a key early indicator of aberrant gene expression in gestational tissues, and thus the basis for new approaches for the early identification of complications of pregnancy, and perhaps for interventions that target aberrant epigenetic status.

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Disclosure

The authors report no conflicts of interest.

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