Review

Regulation of major histocompatibility complex class II gene expression in trophoblast cells

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Abstract

Trophoblast cells are unique because they are one of the few mammalian cell types that do not express major histocompatibility complex (MHC) class II antigens, either constitutively or after exposure to IFN-γ. The absence of MHC class II antigen expression on trophoblast cells has been postulated to be one of the essential mechanisms by which the semi-allogeneic fetus evades immune rejection reactions by the maternal immune system. Consistent with this hypothesis, trophoblast cells from the placentas of women suffering from chronic inflammation of unknown etiology and spontaneous recurrent miscarriages have been reported to aberrantly express MHC class II antigens. The lack of MHC class II antigen expression on trophoblast cells is due to silencing of expression of the class II transactivator (CIITA), a transacting factor that is essential for constitutive and IFN-γ-inducible MHC class II gene transcription. Transfection of trophoblast cells with CIITA expression vectors activates both MHC class II and class Ia antigen expression, which confers on trophoblast cells both the ability to activate helper T cells, and sensitivity to lysis by cytotoxic T lymphocytes. Collectively, these studies strongly suggest that stringent silencing of CIITA (and therefore MHC class II) gene expression in trophoblast cells is critical for the prevention of immune rejection responses against the fetus by the maternal immune system. The focus of this review is to summarize studies examining the novel mechanisms by which CIITA is silenced in trophoblast cells. The elucidation of the silencing of CIITA in trophoblast cells may shed light on how the semi-allogeneic fetus evades immune rejection by the maternal immune system during pregnancy.

Introduction

The successful maintenance of the semi-allogeneic mammalian fetus during pregnancy provides an apparent immunological paradox. Despite the fact that the fetus expresses proteins encoded by paternally-inherited genes, the maternal immune system does not mount an allogeneic rejection response against the fetus. However, fetal or paternal tissues transplanted into the mother at sites other than the uterus are efficiently rejected, which suggests that

the pregnant uterus is an immunologically privileged site [1]. Although the precise mechanisms underlying the lack of a rejection response against the developing fetus remain incompletely understood, trophoblast cells clearly play an essential role in the prevention of these deleterious immune reactions by several mechanisms. These include the production of multiple soluble and membrane-bound factors that inhibit lymphocytic function, and the absence on the trophoblast cell surface of major

histocompatibility complex (MHC) molecules, which are required for immune recognition and activation. This review will focus on the novel mechanisms underlying the silencing of MHC class II molecule expression on trophoblast cells.

Significance of MHC class II antigens and regulation of expression

MHC class II antigens are heterodimeric cell surface glycoproteins that are essential for the activation of CD4+ helper T (T$_H$) lymphocytes, and the subsequent initiation of antibody- and cytotoxic T lymphocyte (CTL)-based immune responses [2]. Antigenic peptides bound to MHC class II molecules on the surface of antigen presenting cells are recognized by T cell receptors (TCR) on antigen-specific T$_H$ cells. The engagement of antigenic peptide: MHC class II complexes with TCRs on T$_H$ cells results in activation and clonal expansion of these cells, which subsequently synthesize and secrete cytokines that activate CTLs or induce the production of antibodies by B cells. Importantly, allelic differences in MHC class I and class II antigens between donor and recipient are the primary molecular basis for immunological rejection of transplanted organs, for rejection can occur when MHC class I or class II antigens on the transplanted organ differing from the recipient are recognized as "non-self" by the recipient's immune system [2].

Trophoblast cells are one of the few cell types that do not express MHC class II or class Ia antigens, either constitutively or after exposure to IFN-γ [3-9]. Understanding the mechanisms by which MHC class II gene expression is silenced in trophoblast cells is of intense interest because of the potential role that the lack of expression of these antigens plays in protection of the fetus from maternal immune system attack. Inappropriate MHC class II antigen expression on trophoblast cells could lead to maternal immune responses against the fetus by either stimulation of allogeneic rejection reactions against trophoblast expressing paternally-derived MHC class II antigens, or by direct presentation of fetally-derived peptides to maternal helper T cells by MHC class II-positive trophoblast cells. Consistent with these possibilities, human choriocarcinoma cell lines engineered to express MHC class II antigens were able to stimulate allogeneic T cell responses, and present antigen to antigen-specific T cells [10,11]. Furthermore, trophoblast cells from the placentas of women suffering from chronic inflammation of unknown etiology and spontaneous recurrent miscarriages have been reported to aberrantly express MHC class II antigens [12,13]. Collectively, these results suggest that stringent silencing of MHC class II antigen expression on trophoblast cells may be critical for the survival of the fetus during pregnancy. It is possible, however, that under certain conditions MHC class II expression is induced in the trophob-

last layer following mechanical injury as described by Sims et al. [14], or during infection by specific pathogens. Interestingly, MHC class II expression is activated in mouse trophoblast cells treated with the histone deacetylase inhibitor trichostatin A (TSA), which is a fungal product [15]. Thus, it is intriguing to speculate that the presence of fungi in the reproductive tract during pregnancy may result in activation of aberrant MHC class II molecule expression on trophoblast cells, which could potentially lead to the generation of a deleterious anti-fetal immune response. Surprisingly, despite the long-standing and widespread belief that aberrant expression of MHC class II antigens on trophoblast cells would lead to immune rejection of the fetus, studies that directly test this hypothesis have not been reported in the literature.

The expression of MHC class II antigens is regulated primarily at the level of transcription, in a developmental and tissue-specific manner. Constitutive MHC class II expression is restricted to professional antigen presenting cells (APC), which include dendritic cells, macrophages, thymic epithelial cells and mature B cells [16]. MHC class II gene expression is downregulated as B cells differentiate into plasma cells [16]. In contrast to APC, fibroblasts and cells of endothelial and epithelial origin do not express class II antigens constitutively, but do so following exposure to IFN-γ [16]. Finally, trophoblast cells, sensory neurons and plasma cells do not express MHC class II antigens, either constitutively or after exposure to IFN-γ [16].

The developmental and cell-type specific pattern of MHC class II gene expression is due to differential expression of a transacting factor termed the class II transactivator (CIITA), which is considered to be the "master regulator" of MHC class II gene transcription [17,18]. Expression of CIITA is constitutive in mature B cells and dendritic cells, and is activated by IFN-γ in fibroblasts, epithelial and endothelial cells [19-21]. The downregulation of MHC class II transcription during differentiation of B cells into plasma cells results from silencing of CIITA expression [22]. Furthermore, the inability of trophoblast cells to transcribe MHC class II genes, even in the presence of IFN-γ, is due to silencing of CIITA expression [11,23,24]. Transfection of CIITA expression vectors into trophoblast cells, plasma cells, and IFN-γ-inducible cells results in constitutive cell surface expression of MHC class II molecules [11,19-24]. Therefore, efforts to understand the cell type specific expression of MHC class II antigens have focused on delineating the mechanisms by which CIITA expression is controlled.

In addition to activating MHC class II genes, CIITA also plays an ancillary role in activating transcription of MHC class I genes in some cell types [25,26]. Importantly,

transfection of JEG-3 choriocarcinoma cells with CIITA expression vectors resulted in the activation of expression of both MHC class II molecules [11,23,24] and the MHC class I molecules HLA-B and HLA-C [11]. MHC class II expression on the JEG-3 cells conferred the ability to activate antigen-specific T cells [11]. Furthermore, JEG-3 cells expressing CIITA were sensitive to MHC-restricted lysis by cytotoxic T lymphocytes [11]. These studies are consistent with the hypothesis that inappropriate expression of CIITA in trophoblast cells can lead to deleterious maternal immune responses against the conceptus.

Regulation of CIITA gene expression in B cells, dendritic cells and IFN-γ-inducible cells

The regulation of constitutive and IFN-γ-inducible CIITA gene expression is controlled predominantly at the level of transcription by four different promoters in human (and three in mice) that function in a cell-type specific manner [27-32]. Constitutive transcription is mediated by the type I promoter in dendritic cells and macrophages, and by the type III promoter in B and activated T cells [27-30]. The cell type specificity of the type II promoter, which is unique to humans, is currently unclear [27]. IFN-γ-inducible CIITA transcription is controlled primarily at the type IV promoter, although the type III promoter is weakly IFN-γ-responsive in select cell types [27,28,31,32]. Since the activity of CIITA promoters I and III is restricted to antigen presenting cells such as dendritic cells and B cells, investigators studying the regulation of CIITA expression in trophoblasts have concentrated on determining the mechanisms involved in silencing from the IFN-γ-responsive type IV promoter (PIV).

IFN-γ-inducible transcription from the CIITA PIV is mediated by the Janus Kinase-1/Signal Transducers and Activators of Transcription-1 (JAK-1/STAT-1) signaling pathway [Figure 1, based on [27,28,31-33]]. Following binding of IFN-γ to its cognate receptor, JAK-1 becomes activated, and it phosphorylates STAT-1 that is localized in the cytoplasm [34]. Phosphorylated STAT-1 subsequently homodimerizes and translocates to the nucleus, where it activates transcription of the gene encoding a transcription factor termed interferon regulatory factor-1 (IRF-1), and a host of other genes including CIITA [27,31-34]. The CIITA PIV contains three conserved elements, including a GAS element, which binds to activated STAT-1, an E box, which binds to the ubiquitously expressed transcription factor USF-1, and an interferon regulatory element (IRE), which binds to IRF-1 and IRF-2 [27,31,32]. Mutational analyses demonstrated that the GAS, E box, and IRE are all required for IFN-γ-inducible CIITA transcription [31,32]. Consistent with these results, IFN-γ-responsive CIITA (and class II) expression is abrogated in both IRF-1 and STAT-1 deficient cells [31,33]. Moreover, the initiation and maintenance of CIITA transcription requires sus-

tained STAT-1 activation and threshold levels of IRF-1 [35]. Interestingly, the transcription factor IRF-2, which is constitutively expressed and normally acts as an antagonist to IRF-1, also appears to play an essential role in IFN-γ-inducible CIITA transcription [36-38].

Recent studies indicate that chromatin structure also plays a crucial role in the transcriptional regulation of CIITA. For instance, IFN-γ-inducible CIITA transcription requires the recruitment and activity of the chromatin remodeling complex SWI/SNF to the CIITA type IV promoter [39]. In addition, studies utilizing chromatin immunoprecipitation assays revealed that acetylation of histones H3 and H4 occurs at the CIITA PIV following exposure of fibroblast and epithelial cells to IFN-γ, and that histone acetylation is associated with successful IFN-γ-inducible CIITA transcription [40]. It is currently unclear how histone acetyltransferases (HATs) are recruited to the CIITA promoter following IFN-γ treatment. One possibility is that STAT-1 and IRF-1 recruit the HATs CBP, pCAF, and/or 300 to the CIITA promoter, for physical interactions between STAT-1, IRF-1 and these HATs have been detected at other IFN-γ-responsive promoters [41]. This is consistent with studies demonstrating that the increase in histone acetylation at the CIITA promoter is coincident with binding of STAT-1 in IFN-γ-treated cells [40]. In summary, studies to date indicate that successful IFN-γ-inducible CIITA transcription requires: 1) sufficient and sustained levels of activated STAT-1, IRF-1 and IRF-2, 2) acetylation of histones at the CIITA PIV, and 3) chromatin remodeling by the SWI/SNF complex.

Regulation of CIITA gene expression in trophoblast cells

As mentioned previously, the inability of human, mouse and rat trophoblast cells to express MHC class II molecules in response to IFN-γ is due to the silencing of CIITA transcription [11,23,24]. Transient transfection assays demonstrated that the IFN-γ-responsive CIITA type IV promoter is functionally active in trophoblast cells, despite the fact that the endogenous CIITA gene is not expressed [11,15,42]. These results suggested that trophoblast cells contain the transacting DNA binding factors (STAT-1, USF-1 and IRF-1) required for IFN-γ-inducible CIITA transcription. Furthermore, these data are consistent with a model in which endogenous CIITA transcription in trophoblast cells is repressed by epigenetic mechanism(s) which maintain a closed chromatin conformation, such as promoter methylation and/or the insufficient acetylation of histones. Epigenetic repression is also consistent with the following observations: 1) neither STAT-1 nor IRF-1 are bound to the GAS and IRE of the CIITA PIV in IFN-γ-treated Jar and JEG-3 choriocarcinoma cells, and 2) the histones H3 and H4 are not acetylated at
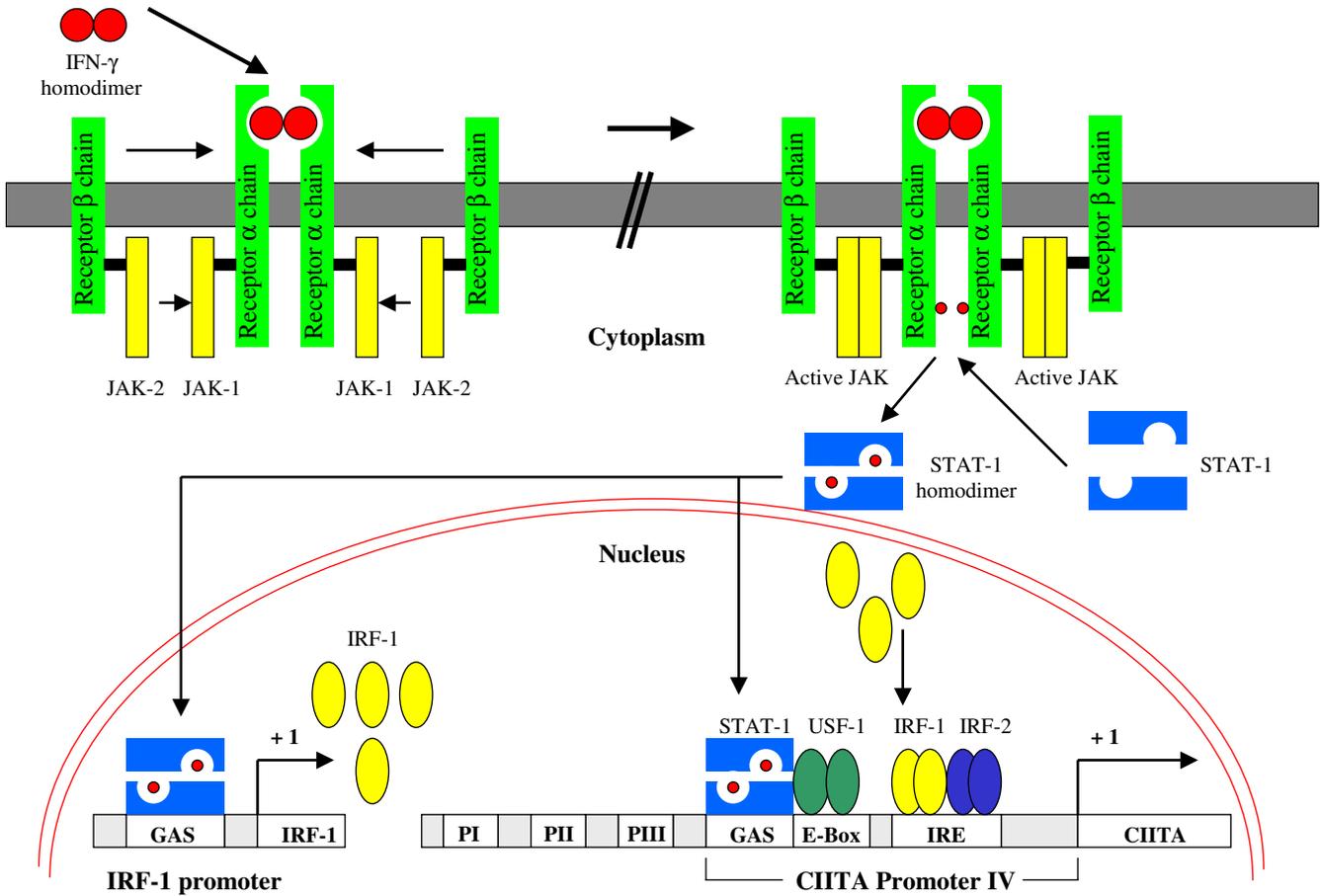
Figure 1
IFN-γ-inducible CIITA transcription from the type IV promoter is mediated by the Janus Kinase-1/Signal Transducers and Activators of Transcription-1 (JAK-1/STAT-1) signaling pathway. Binding of IFN-γ to the IFN-γ receptor (IFN-γ-R) results in activation of the kinases JAK-1 and JAK-2. These kinases subsequently phosphorylate the IFN-γ-R, which leads to a conformational change in the receptor that provides a docking site for monomers of the transcription factor STAT-1 that are localized in the cytoplasm. These STAT-1 monomers are phosphorylated by the JAKs, which leads to dimerization and translocation to the nucleus. STAT-1 activates transcription of a variety of genes, including the transacting factor interferon regulatory factor-1 (IRF-1), by binding to γ-activating sequences (GAS) within the promoters of the target genes. Phosphorylated STAT-1, IRF-1, and the ubiquitously expressed factors USF-1 and IRF-2 subsequently cooperate to activate CIITA transcription by binding to the GAS, E box and interferon responsive element (IRE), respectively, in the CIITA type IV promoter.

the CIITA PIV in these choriocarcinoma cells following exposure to IFN-γ [40,42].

The CIITA type IV promoter was shown to be methylated in Jar and JEG-3 cells [11,15,42,43], and in human chorionic villous trophoblast cells isolated from first trimester placentas [11,44]. Moreover, Morris et al. [42] and van der Stoep et al. [44] reported that Jar and JEG-3 cells treated

sequentially with the demethylating agent 5-azacytidine followed by IFN-γ expressed low levels of CIITA and MHC class II mRNA. These collective results led to the hypothesis that methylation of CIITA PIV is responsible for repression of IFN-γ-inducible CIITA transcription in trophoblast cells. Contrary to these studies, recent work from our laboratory demonstrated that the CIITA PIV is not methylated in primary human cytotrophoblast cells freshly

isolated from term placentas, or in mouse or rat trophoblast cell lines. In addition, CIITA PIV is methylated in human 2fTGH fibrosarcoma cells, which are IFN-γ-inducible for CIITA expression [15]. Based on the studies by van den Elsen's group [44] and our group [15], it is possible that the methylation status of CIITA PIV in human trophoblast cells may vary during different stages of pregnancy. However, the collective results from our laboratory [15] strongly suggest that silencing of IFN-γ-inducible CIITA transcription in trophoblast cells is not due to methylation of the CIITA PIV.

CIITA gene expression is activated in mouse trophoblast cell lines treated with the combination of IFN-γ and the histone deacetylase (HDAC) inhibitor trichostatin A (TSA) [15], which indicates that HDACs are involved in silencing CIITA transcription in these cells. Furthermore, the demonstration that histones H3 and H4 are not acetylated at the CIITA PIV in Jar or JEG-3 choriocarcinoma cells after exposure to IFN-γ [40] suggest that the appropriate histone acetyltransferases (HATs) are either insufficiently expressed, and/or not effectively recruited to the CIITA PIV in trophoblast cells exposed to IFN-γ. Since acetylation is closely associated with an open chromatin conformation and successful transcription, these results support the concept that CIITA transcription is silenced in trophoblast cells by an epigenetic mechanism that results in a closed chromatin conformation at CIITA PIV.

As mentioned previously, the CIITA PIV is inducible by IFN-γ in transient transfection assays of trophoblast cells, which suggests that these cells contain the transacting factors (STAT-1, USF-1, IRF-1 and IRF-2) required for IFN-γ-inducible CIITA transcription. However, recent data from our laboratory demonstrate that although basal STAT-1 expression is comparable in human trophoblast versus HeLa cells, the IFN-γ induced levels of the STAT-1, IRF-1, and IRF-2 transcription factors are significantly reduced in trophoblast cells relative to HeLa or 2fTGH fibrosarcoma cells [45]. Similar results were observed in studies of mouse trophoblast cell lines [46]. These collective results can be interpreted at least two different ways: 1) trophoblast cells express sufficient levels of activated STAT-1, IRF-1 and IRF-2 to drive IFN-γ-inducible transcription from CIITA PIV; or 2) the chromatin structure may affect the relative amounts of STAT-1 and IRFs required to activate the CIITA PIV. In the latter instance, the endogenous PIV may require higher levels of the transcription factors for activation than the transfected, unintegrated CIITA PIV. The reduced levels of STAT-1 and IRF-1 in IFN-γ-treated trophoblast cells may also help account for: 1) why binding of these factors to the CIITA PIV has not been detected by *in vivo* genomic footprinting or chromatin immunoprecipitation assays of trophoblast cells [40-42], and 2) the lack of histone acetylation at the CIITA promoter in these

cells, for both STAT-1 and IRF-1 recruit histone acetyltransferases (HATs) to IFN-γ-responsive promoters [41]. Further studies are clearly required to address these issues.

A novel method for actively repressing CIITA transcription in trophoblast cells was proposed based on studies in which HeLa cells (which express CIITA in response to IFN-γ) were transfected with a Jar cell cDNA expression library, and negatively selected for MHC class II expression [47-49]. An untranslated poly A+ RNA (utRNA) was identified in choriocarcinoma cells that could repress CIITA and MHC class II expression when transfected into either HeLa cells or CH27 mouse B cells. However, the precise mechanism by which this utRNA may suppress CIITA transcription is currently unclear. Peyman [47] reported that this RNA contains STAT-1 (GAS) binding motifs, and proposed that by competitively binding to activated STAT-1 in the cytoplasm, the utRNA blocks STAT-1 nuclear translocation and therefore IFN-γ-inducible gene transcription. In contrast, Geirsson et al. [48] stated that this utRNA blocked CIITA type IV promoter activity in transient transfection assays of HeLa cells, and that the sequences of the CIITA PIV between the GAS and IRE were required for transcriptional repression. These results appear to contrast with the work of three other groups [11,15,42] showing that the CIITA PIV is active in transient transfection assays of trophoblast cells. In a separate study, Geirsson et al. [49] also showed that this trophoblast utRNA inhibited transcription from the B cell-specific CIITA type III promoter when transfected into CH27 murine B cells. The CIITA type III promoter fragment utilized in these studies does not contain a GAS, and the specific CIITA PIII sequences required for transcriptional repression were not identified in this study. Thus, further studies are necessary to elucidate the precise role that this utRNA may play in silencing CIITA transcription in trophoblast cells.

As an additional means to investigate the genetic mechanisms regulating CIITA and MHC class II gene expression in trophoblast, stable heterokaryons were generated between trophoblast and CIITA-expressing cells [15,50]. Neither CIITA nor MHC class II molecules were expressed in stable hybrids between Jar cells and B cells, suggesting that silencing of transcription from the B cell-specific CIITA PIII is dominant in trophoblast cells [50]. In contrast, hybrids between human Jar or JEG-3 cells and 2fTGH fibrosarcoma cells expressed both CIITA and MHC class II following exposure to IFN-γ [15], indicating that silencing of IFN-γ-inducible transcription from the CIITA PIV in trophoblast cells is recessive. Identical results were obtained in studies of hybrids between mouse M-11 trophoblast cells and NIH-3T3 fibroblasts [15]. One explanation for the recessive nature of the silencing of IFN-γ-inducible CIITA transcription is that trophoblast cells express insufficient levels of essential transacting factor(s),
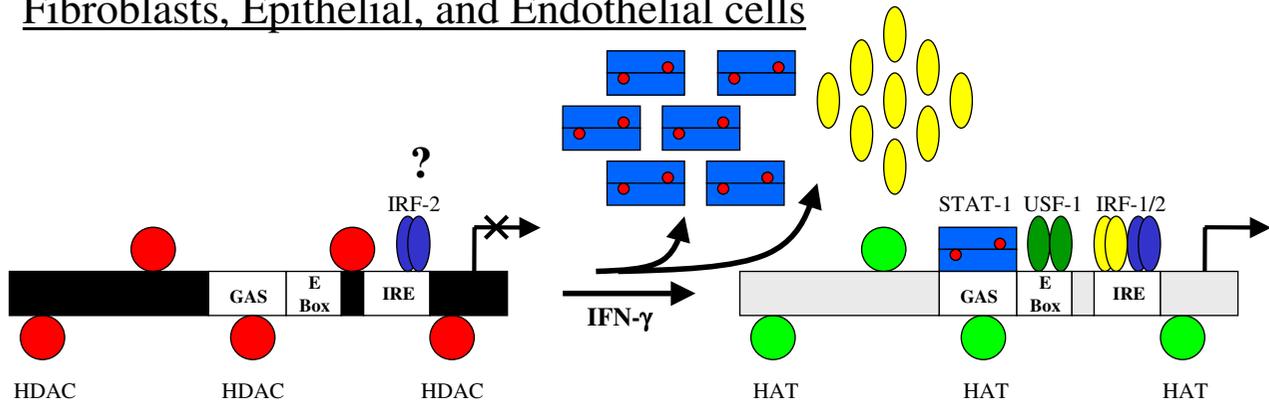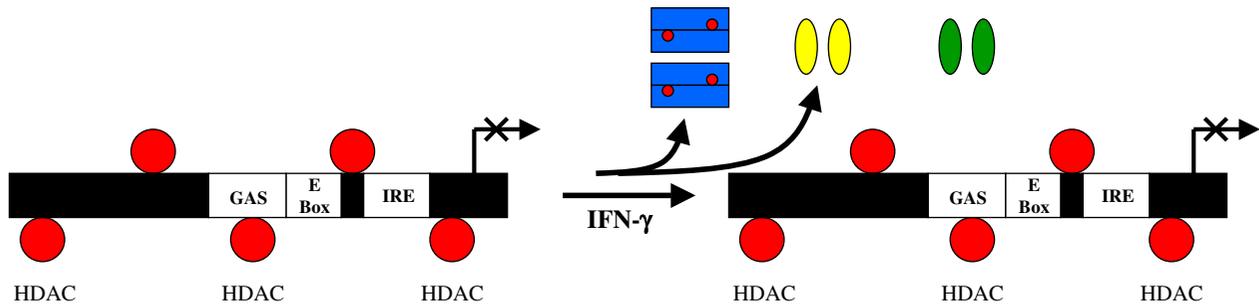
Figure 2
Schematic of a general model for the silencing of CIITA transcription in trophoblasts. The CIITA PIV is associated with HDACs (red circles) in CIITA-inducible cells and trophoblasts in the absence of IFN-γ, which helps to maintain a closed chromatin conformation (represented as blackened bars). IRF-2 may or may not be bound constitutively to the CIITA PIV in CIITA-inducible cells. Following exposure of the cells to IFN-γ, STAT-1 (blue rectangle) is phosphorylated (represented by the red dot), and subsequently dimerizes, translocates to the nucleus, and induces synthesis of IRF-1 (yellow ovals), but the levels of these factors are higher in CIITA-inducible cells relative to trophoblasts. STAT-1 and IRF-1 bind to their respective elements at the PIV in CIITA-inducible cells, and recruit HATs (light green circles) to the promoter. This leads to changes in the chromatin to an open conformation (light gray bars) and initiation of transcription. In contrast, this sequence of events does not occur in trophoblasts, and the chromatin remains in a closed conformation.

and that higher level expression of these factors is dominant in the stable hybrids. Interestingly, the levels of IRF-1, IRF-2 and STAT-1 expression in the trophoblast/ fibroblast hybrids were similar to the parental fibroblasts [44,45], which is consistent with, but does not prove, the possibility that the failure of trophoblast cells to express CIITA may in part be due to sub-threshold levels of these DNA binding proteins. Alternatively, trophoblast cells may have deficiencies in factor(s) such as HAT(s), that

plays a crucial role in controlling the chromatin structure at the CIITA type IV promoter, since histones are not acetylated at the CIITA promoter in Jar or JEG-3 cells [40] and TSA partially alleviates silencing of IFN-γ-inducible CIITA expression in mouse trophoblast cells [15]. Lastly, trophoblast cells may contain a factor(s) that represses CIITA transcription, perhaps by recruiting histone deacetylases. In this scenario, expression of this putative repressor factor would be extinguished in the hybrids. It

remains to be determined whether this is the case for the trophoblast-specific utRNA described by Peyman [47] and Geirsson et al. [48,49].

Conclusions

Silencing of CIITA transcription in trophoblast cells is likely to be crucial for successful maintenance of the fetus, and recent studies suggest that the precise mechanism of silencing may be novel. Therefore, we propose the following general model to describe the mechanism by which CIITA is silenced in trophoblast (Figure 2). In the absence of IFN-γ, the CIITA PIV is associated with histone deacetylases (HDACs) and is therefore in a closed chromatin conformation in CIITA-inducible cells (fibroblasts, epithelial and endothelial cells), and trophoblast cells. The precise chromatin conformation of PIV may differ between trophoblast and the CIITA-inducible cell types because IRF-2, which is constitutively expressed at higher levels in CIITA-inducible cells compared to trophoblast cells [45], may be bound to the IRE in CIITA-inducible cells prior to IFN-γ treatment. Following exposure to IFN-γ, STAT-1 is activated, and IRF-1 synthesized in all of the cell types, but the levels of these factors are reduced in trophoblast cells relative to CIITA-inducible cells. These factors successfully bind to their respective elements at the CIITA PIV in fibroblasts, epithelial and endothelial cells, but fail to do so stably in trophoblast. Histone acetyltransferases (HATs) are subsequently recruited to the CIITA PIV by STAT-1 and the IRFs in CIITA-inducible cells, and HDACs are displaced, which results in further "opening" of the chromatin, and induction of CIITA transcription. In contrast, HDACs remain associated with PIV in trophoblast cells because the HATs are not recruited, and the CIITA PIV chromatin conformation remains closed.

The elucidation of the silencing of CIITA in trophoblast cells will shed light on one of the novel mechanisms by which the fetus may evade rejection by the maternal immune system during pregnancy. These studies may also have important implications for tumor immunity, for they will enable us to determine whether tumor cells that fail to express CIITA (and hence MHC class II) are usurping normal cellular mechanisms of gene regulation to evade host immune responses. Lastly, these studies may have an impact on autoimmunity and transplantation, for aberrant expression of MHC class II antigens is associated with certain autoimmune disorders, and class II antigens are one of the targets in transplant rejection.

Acknowledgments

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