CHARACTERIZATION OF EPIGENETIC MECHANISMS INVOLVED IN EMBRYO IMPLANTATION (DNA METHYLATION GOVERNED EXPRESSION OF THE NOVEL TEAD4 PROMOTER)

Ph.D. Thesis

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1. INTRODUCTION

One in seven couples worldwide suffer from infertility, and failure to implant an embryo is a major cause of unwanted childlessness. Human embryo implantation is an essential spatial and temporal process for successful pregnancy. The role of epigenetic factors and processes in endometrial receptivity is also essential. Epigenetic modifications, including DNA methylation, various histone modifications, and microRNAs, are involved in the development of the appropriate gene expression profile of the embryo and endometrium. The work presented here describes the discovery of an alternative isoform of the TEAD4 transcription factor, which is putatively required for trophectoderm lineage differentiation and subsequent embryonic implantation. Our experiments have investigated the role of DNA methylation, one of the best known epigenetic mechanisms, in regulating the expression of the novel TEAD4 isoform in certain tissues, including the umbilical cord and placenta.

1.1. Epigenetics

A growing body of evidence shows that, in addition to the inherited genetic make-up (i.e. genomic DNA), various environmental factors also contribute significantly to the etiology of disease. Epigenetic mechanisms respond to external stimuli and act as a bridge between the environment and the DNA carrying genetic information. Epigenetic mechanisms help interpret genetic information by regulating gene expression and thereby influence cellular activity. Overall, epigenetic mechanisms increase the complexity of most disorders by providing subtle contributions to gene expression. Although there is controversy about the involvement of genetic and epigenetic factors in disease etiology, it is becoming increasingly clear that these two systems interact and are ultimately responsible for the development of some of the most complex diseases. Epigenetics originally focused on DNA methylation and various histone modifications, but has recently expanded to include non-coding RNAs. Ab ovo, all cells in the body inherit the same genetic information. What makes each cell unique is that different sets of genes are switched on and off during ontogenesis. Epigenetics - in a broad sense, a bridge between genotype and phenotype - is a phenomenon that alters gene expression in a particular chromosomal region without changing the primary nucleotide sequence. Epigenetic mechanisms determine the expression of cell-specific genes and are responsible for cellular memory, i.e. the maintenance and transmission of cell-specific gene expression patterns to progeny cells. Epigenetic factors are capable of generating, interpreting and deleting epigenetic information, and in this sense can be divided into different functional groups: epigenetic "writers" or enzymes that modify DNA and histones; epigenetic "readers" with specific domains that recognize DNA or histone signals; and epigenetic "erasers" that can delete existing signals to make place for new modifications.

1.1.1. Writers of DNA methylation

DNA methyltransferases (DNMTs) are a family of enzymes responsible for writing and maintaining DNA methylation. These enzymes specifically recognise cytosines and transfer methyl groups from S-adenosylmethionine (SAM) to target DNA sequences. DNA methylation occurs at the C5 position of CpG dinucleotides and is catalysed by two main classes of enzymes -

maintenance methylation and de novo methylation. DNMT1 is the maintenance methyltransferase responsible for the transfer of DNA methylation patterns to daughter strands during DNA replication. Without DNMT1, unmethylated daughter strands would be generated, leading to passive demethylation and genome instability. DNMT3a and DNMT3b can be considered as de novo methyltransferases that establish the DNA methylation pattern during early development.

1.1.2. Readers of DNA methylation

Understanding the function and importance of DNA methylation in gene expression and cell differentiation requires a detailed study of the proteins that read methylated signals. These proteins, broadly referred to as 'readers' of DNA methylation, are crucial for the conversion of epigenetic signals into biological outcomes, primarily in gene repression, but also in activation in certain contexts. The primary group of proteins involved in reading methylated DNA are methyl-CpG-binding domain (MBD) proteins. This family comprises several key members, MeCP2, MBD1, MBD2, MBD3 and MBD4, each with unique binding properties and biological functions, but generally for the recognition and binding of methylated CpG dinucleotides within DNA. MeCP2 is perhaps the best studied because of its clinical relevance; mutations in the MeCP2 gene are the primary cause of Rett syndrome, a severe neurological disorder. Functionally, MeCP2 binds to methylated DNA and recruits other chromatin remodelling proteins that contribute to chromatin compaction, leading to gene silencing. MBD1 plays a role in maintaining genome stability and regulating gene transcription through its interaction with chromatin modifiers and corepressors. It binds preferentially to methylated DNA and recruits H3 histone H3 lysine 9 (H3K9) methyltransferases, confirming the transcriptionally inactive state of chromatin. MBD2 and MBD3 do not bind as tightly to methylated DNA as MeCP2 or MBD1, but are part of the NuRD complex, which plays a major role in chromatin remodelling and transcriptional repression. MBD2 has been shown to be essential in mediating the effects of DNA methylation on gene silencing. MBD4 has a slightly different role, primarily involved in DNA repair. It binds to methylated DNA at sites of cytosine deamination, recognises thymine-guanine mismatches and initiates repair processes to prevent mutations, thus maintaining genome integrity. In addition to the MBD family, another major reader of methylated DNA is UHRF1 (Ubiquitin-like with PHD and RING Finger domains 1), which plays a key role in maintaining DNA methylation during DNA replication. UHRF1 recognizes haemimethylated DNA and recruits DNMT1 to these sites, ensuring that the newly synthesized DNA strand has the same methylation pattern as the parent strand.

1.1.3. Erasures of DNA methylation

DNA methylation is a reversible epigenetic modification, and the enzymes responsible for removing methyl groups from DNA - the "deletion enzymes" - are crucial for the dynamic change in gene expression. DNA demethylation can occur by passive and active demethylation, which are due to different enzyme activities. Passive demethylation occurs during DNA replication. This process results in the dilution of the methyl signal due to the absence or reduced activity of DNA methyltransferases (DNMTs), in particular DNMT1, which is thus unable to maintain methylation

patterns during cell division. Active demethylation, on the other hand, is a more direct and rapid enzymatic process involving several key players, in particular the Ten-Eleven Translocation (TET) family of enzymes. These enzymes - TET1, TET2 and TET3 - catalyse the oxidation of 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC), an intermediate step towards complete demethylation. This conversion is crucial, as 5-hmC is further oxidized to form either 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC), which can be excised by thymine-DNA glycosylase (TDG) and then replaced by unmodified cytosine via the base excision repair (BER) pathway. TET enzymes not only promote active demethylation but also serve as epigenetic markers in their own right. 5-hmC, for example, is enriched in gene bodies and enhancers, particularly in brain and embryonic stem cells, suggesting a role in the regulation of active transcription and pluripotency.

1.1.4. DNA methylation changes in the early stages of animal development

After fertilisation of the egg, a single-celled zygote is formed, which undergoes several fission cycles without increasing the volume of the entire embryo. As the embryo reaches the eight-cell stage, polarization allows compression, which paves the way for the formation of distinct cell lines: the trophectoderm (TE) and the intracellular mass (ICM). These cell lines develop during the subsequent asymmetric cleavage divisions and continue until the formation of the blastocoel cavity. Cavity formation results in the full expansion of the blastocyst, which is released from the surrounding zona pellucida layer before embedding in the uterine lining. Dynamic changes in DNA methylation occur during preimplantation embryogenesis. After fertilisation, the paternal and maternal nuclei are formed and undergo active and passive demethylation. As embryonic development progresses, de novo methylation occurs, which is crucial for the first cell differentiation and silencing of pluripotency-sustaining genes. Despite global fluctuations in methylation levels, the methylation status of imprinted genes remains consistent. Studies have shown that Dnmt1 can maintain methylation in most imprinted loci without the help of Dnmt3a and Dnmt3b. In addition, the Dnmt1s isoform is linked to the nucleus throughout preimplantation development, helping to maintain methylation in specific genomic regions. Methylation also varies beyond the global level; Dnmt1o, a Dnmt1 isoform that is primarily expressed during oogenesis and early preimplantation, shows stage-specific methylation changes. Analysis of the expression pattern of methyltransferases and their interacting proteins indicates distinct epigenetic profiles within a single embryo. This complexity requires detailed single-cell methylation profiling to distinguish cell fates and contributes to understanding the complex process of DNA methylation reprogramming during early development. DNA methylation is thus a fundamental and dynamic epigenetic modification that is essential for preimplantation embryonic development, affecting both global and locus-specific gene expression and highlighting the need for detailed epigenetic profiling at the single-cell level.

1.2. TEAD transcription factor family

The TEAD family consists of four different members (TEAD1-4), encoded by four separate genes, and is expressed in almost all mammalian tissues. The N-terminal domain binds to DNA cis-

elements such as the 5′- GGAATG - 3′ sequence present in the SV40 enhancer and promoter regions of the TEAD target gene. The C-terminus functions as a transactivation domain in the recruitment of transcriptional coactivators. Several coactivator candidates of TEADs have been identified, including YAP and its paralog, TAZ, vgll proteins and the p160 family of nuclear receptor coactivators. TEAD1 plays a key role in myocardial development by promoting the expression of heart-specific genes. The role of TEAD2 remains somewhat unclear, but it is thought to be involved in the regulation of brain development. TEAD4 is mainly involved in embryo implantation. The precise function of TEAD3 is still under investigation.

1.3. The TEAD4 protein and Hippo signaling

TEAD transcription factors are key mediators of the Hippo signaling pathway. They exert their activity through interactions with nuclear coactivators, which are classified into three groups: YAP (yes associated protein) and its paralog TAZ (transcriptional coactivator with PDZ-binding motif, also known as WWTR1), VgLLs and p160s proteins. The Hippo signaling pathway is a regulator of organ size that acts through a central kinase cascade. YAP or TAZ, its downstream effectors, are located in the nucleus unphosphorylated and translocate to the cytoplasm upon phosphorylation. In their phosphorylated form, YAP and TAZ are degraded via the ubiquitin/proteasomal pathway. In their unphosphorylated form, YAP/TAZ are translocated to the nucleus and activate various nuclear transcription factors, including TEADs. There is increasing evidence that some key components of the Hippo-YAP pathway are regulated at the level of alternative splicing. For example, YAP has eight different splicing isoforms. The role of these isoforms is not yet fully understood. Since TEAD4 is a key component of Hippo signaling, it is hypothesized that additional isoforms may exist and play a biologically relevant role in this process. TEAD4 plays a critical role in the Hippo signaling pathway. The regulation of TEAD4 in this pathway depends on the phosphorylation status of its coactivators, Yes-associated protein (YAP) and the transcriptional coactivator with PDZ-binding motif (TAZ). In addition, TEAD4 plays a key role in embryonic development and pluripotency as part of the transcriptional network that regulates pluripotency of embryonic stem cells. It interacts with essential pluripotency factors such as Oct4, Sox2 and Nanog and regulates genes that are essential for maintaining embryonic stem cells in an undifferentiated state. This interaction highlights the importance of TEAD4 in early developmental stages and cell differentiation. TEAD4 also plays a role in the Wnt signaling pathway, which is crucial for cell fate determination and maintenance of tissue homeostasis. TEAD4 can interact with β-catenin, a key mediator of Wnt signalling, to regulate gene expression. Such interaction between TEAD4 and Wnt signaling underscores the ability of the protein to integrate different signaling pathways, promoting complex cellular responses.

1.4. The role of Teads in embryo development

During pre-implantation development in mice, embryos form blastocysts by forming the first two cell lines: the trophectoderm (TE), which gives rise to the placenta, and the inner cell mass (ICM), which gives rise to the embryo proper. The differentiation of TE is regulated by the Caudal-related homeobox 2 (Cdx2) transcription factor, but the mechanisms that precede Cdx2 expression remain unknown. Tead1, Tead2, and Tead4 have been shown to be expressed in preimplantation embryos,

and at least Tead1 and Tead4 are widely expressed in both TE and ICM lineages. However, embryos expressing Tead4 (Tead4$^{-/-}$) die at the preimplantation stage without developing blastocoel. Furthermore, no TE-specific genes, including Eomes and the Cdx2-independent Fgfr2 gene, are detectable in Tead4$^{-/-}$ embryos. Instead, the ICM-specific transcription factors Oct3/4 and Nanog are expressed in all blastomeres. Tead4$^{-/-}$ embryos also fail to differentiate into trophoblast giant cells when cultured in vitro, although embryonic stem (ES) cells with normal differentiation capacity can still be generated from these embryos. These results suggest that Tead4 has a role in preimplantation TE specification distinct from Tead1 and Tead2, and that Tead4 is an early transcription factor required for trophectoderm lineage development, including expression of Cdx2. Functional redundancy between Tead1 and Tead2 was observed, with Tead1 and Tead2 double mutant embryos showing more severe defects than Tead1 or Tead2 single mutant embryos. However, inactivation of Tead4 in mice severely affects trophectoderm specification, leading to embryo implantation failure, which appears to be a primary function of Tead4. Notably, embryos develop normally when Tead4 function is disrupted after implantation. Initially, it was shown that Tead4 is not required for blastocoel formation when embryos are cultured at low oxygen concentrations. However, Tead4 knockout embryos cultured in the absence of glucose do not induce blastocoel formation, unlike wild-type embryos. This observation suggests that TEAD4 plays a role in the establishment of energy homeostasis during preimplantation development for the transition from morula to blastocyst.

Tead4 also plays a crucial role in post-implantation development by regulating trophoblast progenitor self-renewal and stem development in the placental primordium. In post-implantation mouse embryos, Tead4 is selectively expressed in trophoblast stem cell-like progenitor cells (TSPCs). Loss of Tead4 in post-implantation mouse TSPCs impairs self-renewal, leading to embryonic lethality before embryonic day 9.0 (E9.0), the developmental stage corresponding to the first trimester of human pregnancy. TEAD4 and its cofactor, Yes-associated protein 1 (YAP1), are also specifically expressed in human placental first trimester cytotrophoblast (CTB) progenitors. Some unexplained recurrent pregnancy losses (idiopathic RPL) have been associated with impaired expression of TEAD4 in CTB progenitors. By generating RPL-patient-specific trophoblast stem cells (RPL-TSCs), it has been shown that loss of TEAD4 correlates with defective self-renewal of RPL-TSCs, and that restoration of TEAD4 expression rescues this ability. Global gene expression (RNA-Seq) analysis of Tead4-deficient mouse TSCs, combined with ChIP-Seq data, revealed that Tead4 directly regulates various cell cycle regulators, including several cyclin/CDKs. Tead4-mediated regulation of cyclins/CDKs in primary trophoblast progenitors is essential for their self-renewal and expansion. TEAD4 therefore plays an essential role in trophoblast cell homeostasis during placental development, preventing placental insufficiency and ensuring a successful pregnancy.

2. SPECIFIC AIMS

Transcriptional regulation plays a critical role in embryo implantation in the endometrium. TEAD4 is a transcription factor that plays a pivotal role in the organization of molecular events that are critical for successful embryo implantation in the endometrium. TEAD4 is known to regulate the expression of genes involved in cell adhesion, trophoblast invasion and endometrial receptivity. TEAD4 is also involved in post-implantation development and plays a critical role in placental development and function. Dysregulation of TEAD4 can lead to abnormalities in placental structure and function, resulting in pregnancy complications such as intrauterine growth-uterine insufficiency or preeclampsia. Accordingly, studying the epigenetic context of TEAD4 provides valuable insights into the role of TEAD4 in development, reproduction and disease. Hence, characterisation of TEAD4 may be important for diagnostic and therapeutic purposes in reproductive medicine. The following objectives were set for the analysis of TEAD4 and its potential isoforms:

1) Mapping epigenetic patterns around the TEAD4 gene encoding human chromosome 12.

2) Search for novel promoter regions of TEAD4 that may explain the complex function of TEAD4.

In silico study of epigenetic histone profiles suggested that a novel TEAD4 isoform with unknown function may be present in cells and we wanted to map its expression in tissues:

3) Cloning and nucleotide sequence analysis of the novel TEAD4 isoform.

4) Analysis of the gene expression pattern of the new isoform.

5) Elucidation of the subcellular localization of the novel isoform.

6) Identification, cloning and study of the alternative TEAD4 promoter under transient expression conditions.

Having shown that the new isoform is present in the placenta, we added two new aims to our studies:

7) To demonstrate that the mRNA encoding the new isoform is translated in the placenta.

8) To investigate the DNA methylation pattern in placenta and what role it may play in its expression.

In summary, the main objectives of the PhD thesis were to identify potential novel TEAD4 isoforms, characterise their expression and investigate how epigenetic mechanisms such as DNA methylation may play a role in these processes.

3. RESULTS

3.1. Epigenetic profiles predict a new promoter in the 3rd intron of TEAD4 gene

Our original hypothesis was that the TEAD4 gene may have multiple promoters and that the new transcript encoded isoform may be involved in the implantation of blastocysts into the endometrium. Several cases are known from the literature where different promoters have been used in association with cell type-specific genetic and epigenetic decisions. To explore additional, previously unidentified promoters of the TEAD4 gene, we investigated epigenetic patterns in the TEAD4 gene and adjacent chromosomal regions in the ENCODE database. At the beginning of the in silico analysis, we focused on epigenetic histone marks known to be capable of signalling transcriptionally active promoters. For example, tri-methylation or acetylation on histone 4 (H3K4me3) and histone 27 (H3K27ac) of H3 histone and depoisomerization of the Z isoform of histone 2A (H2A.Z) are indicators of transcriptionally competent promoters. The presence of a transcriptionally active promoter is indicated by the overlap of RNA polymerase II (Pol2) depotentiation with these epigenetic histone signals. We observed overlap of all these signals in the intergenic region of the TEAD4 gene, suggesting the existence of a novel promoter embedded in intron 3. The presence of this hypothetical TEAD4 intronic promoter is supported by the ChIP-Seq data on transcription factors in the ENCODE database. The analysis was performed on two well-known cell lines: K562, a human myelogenous leukaemia-derived cell line, and H1-hESC, a totipotent human embryonic cell line.

Epigenetic signal analysis identified two regions of overlapping epigenetic markers, suggesting strong transcriptional activity. One is the recognised canonical TEAD4 promoter, the other is located approximately 40 kb downstream in intron 3. This predicted intronic predicted promoter has not yet been investigated. The ChIP-Seq data indicate that it is functional only in certain cell types, suggesting that the TEAD4 isoform encoded by transcripts from the alternative TEAD4 promoter may have a specific role in certain cells.

3.2. The presence of the TEAD4 promoter predicted in silico is confirmed by the experimental results.

The transcription initiation point of the predicted promoter was determined by the 5'RACE method using RNA isolated from K562 cells. The resulting fragment was amplified by PCR and incorporated into a plasmid vector (pDrive), and the nucleotide sequence of the cloned fragment was determined by Sanger sequencing. The nucleotide sequence of the fragment was used to identify the transcription start site in the human genome. Accordingly, the new transcript is initiated in intron 3 of the TEAD4 gene, i.e. a new TEAD4 promoter has been identified. Furthermore, given the nucleotide sequence, we designed PCR primers to test which additional exons are carried by the 3' end of the intronic-initiated transcript. The PCR product was cloned and sequenced in a similar manner to the one described above. Analysis of the sequence data indicates that the first exon identified by the new promoter is non-coding, but the additional exons are identical to those of the original TEAD4 gene.

3.3. The Novel TEAD4 Isoform Encodes a DNA-Binding Domain-Less Protein

The newly identified transcript is significantly shorter than the full-length TEAD4 variant, TEAD4-FL. In silico translation of the new transcript shows that it encodes a truncated TEAD4 isoform (TEAD-ΔN) lacking the N-terminal DNA-binding domain, but is identical to the canonical variant in the C-terminal region. Deletion of the DNA-binding domain of the TEAD-ΔN isoform raises intriguing questions about its cellular and molecular functions.

3.4. TEAD-ΔN isoform is excluded from the nucleus

The precise subcellular localization of proteins is determined by different regulatory mechanisms. In many cases, the nuclear localizations signal (NLS) is important for a protein to be translocated to the nucleus, but there are also examples of proteins without NLS translocating to the nucleus. Our hypothesis was that determining the localization of the isoform could provide insight into the cellular function of the new isoform. NLS prediction software has failed to identify NLS in the TEAD-ΔN isoform. To investigate the subcellular localization of the isoform, transient expression experiments were performed. Both isoforms were inserted into plasmids encoding fluorescent proteins. The GFP-encoding plasmid was used to clone full-length TEAD4 (TEAD4-FL), while the RFP-encoding plasmid was used to clone TEAD4-ΔN. These recombinant plasmid constructs were co-transfected into eukaryotic cells and their subcellular localization was monitored by microscopy. The full-length TEAD4 isoform was predominantly localized in the nucleus, whereas truncated TEAD4-ΔN was predominantly observed in the cytoplasm, but a smaller proportion of nuclear localization also occurred.

3.5. TEAD4-ΔN expression is cell type-specific

Isoform-specific PCR was used to investigate the expression of TEAD4 isoforms in different human tissue samples. The full-length TEAD4 isoform was detectable by PCR in all tissues tested. However, expression of the TEAD4-ΔN variant was restricted to certain cell types, which may indicate a specific cell biological role for the newly identified isoform. In addition to RNA expression, the protein expression profile was also examined to exclude the possibility that translational regulation is involved in the regulation of isoform expression. The protein expression profile of TEAD4 isoforms was determined by Western blot analysis on different cell lines . The longer TEAD4 isoform was present in all cell lines tested, which is in agreement with the results of PCR assays. In contrast, the shorter TEAD4 isoform was detected only in certain cell lines and showed lower expression levels compared to the longer form.

3.6. In vitro analysis of the alternative TEAD4 promoter

Part of transcriptional control is that transcription factors (TFs) can interact with cis-elements of promoter and enhancer regions. These interactions promote the formation of preinitiation complexes, leading to efficient transcription by the enzyme RNA polymerase II. Exploration of the TEAD4-ΔN promoter TF binding sites revealed several potential cis-elements, including the consensus motif of TEAD4 itself. To confirm the binding of TEAD4 to the predicted consensus motif within the TEAD4-ΔN promoter, gel-shift or EMSA experiments were performed. Since high-quality TEAD4 antibodies for chromatin immunoprecipitation (ChIP) assays were not

available, we used a competitive EMSA assay to validate the binding of TEAD4 to this promoter region. The TEAD4 cis-DNA element sequence is also very similar to the TEAD4 consensus sequence. Our EMSA results demonstrate that TEAD4 binds to the alternative TEAD4-ΔN promoter and putatively regulates its expression.

3.7. Functional characterization of TEAD4 promoter(s) in transient transfection studies

To deepen our understanding of how TEAD4-ΔN is regulated at the transcriptional level and to test the relevance of our in vitro DNA-protein interaction findings, we PCR-amplified the promoter region upstream of the transcription start site (TSS) and linked it to a luciferase reporter gene. We chose a 1.3-kilobase segment of the intron, as highlighted in the Ensembl Regulatory Build—a detailed repository of epigenetic markers and transcription factors—because it succinctly outlines potential regulatory areas for TEAD4-ΔN promoter region. The promoter region is evolutionarily conserved in mammals, suggesting that it plays a significant role in the regulation of transcription. To analyze its function in a eukaryotic context, we generated recombinant luciferase reporter plasmids containing both the canonical TEAD4 and TEAD4-ΔN promoters and irrespective of luciferase reporter gene expression. These reporter gene constructs were introduced into HEK293 cells. In our assays, the newly identified promoter showed significant luciferase activity, although less intense than the canonical promoter. This suggests that the isoform with the DNA-binding domain may have a more prominent role in cellular function, whereas the TEAD4-ΔN isoform may be important in certain situations and cell types. To investigate the effects of TEAD4 interaction with this promoter region, a TEAD4-overexpressing plasmid was co-transfected with a reporter gene construct containing a 1.3 kb TEAD4-ΔN promoter. We observed that increasing the amount of TEAD4-overexpressing plasmid also increased the promoter activity. This suggests that TEAD4 acts as a positive regulator of truncated isoform expression. However, it is important to note that too high levels of TEAD4 can inhibit promoter function. Taken together, our data show that TEAD4 modulates the expression of its own isoform by interacting with the new intronic promoter.

3.8. TEAD4-ΔN expression in human placenta is regulated by DNA methylation

TEAD4 plays a critical role in ensuring the survival of human embryos after implantation by regulating the self-renewal and development of trophoblast progenitors in the placental primordium. In mice, the lack of TEAD4 in trophoblast stem/progenitor cells (TSPCs) leads to reduced self-renewal capacity after implantation, causing embryo death before embryonic day 9. This developmental stage corresponds to the first trimester of pregnancy in humans. Therefore, understanding the role of TEAD4-ΔN expression at this critical developmental stage is of considerable interest. As a preliminary step, we examined the expression pattern of TEAD4 isoforms in human placental and umbilical cord tissue samples. Our analysis revealed different expression profiles of the two TEAD4 isoforms in human samples. TEAD4-ΔN was absent from umbilical cord samples but was detectable in placental lysates. This differential expression suggests a unique role for the TEAD4-ΔN isoform in placental development. Epigenetic modifications, such as DNA methylation and various histone modifications, create a chromatin

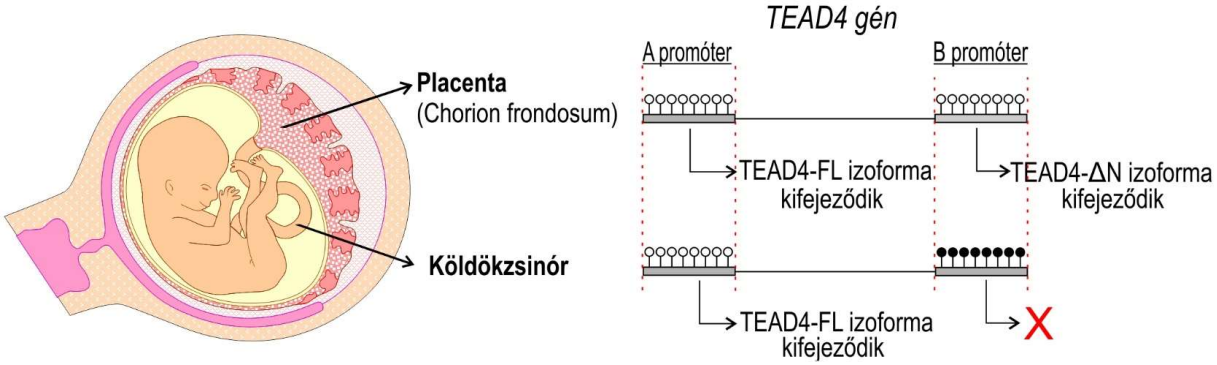
environment that promotes efficient transcription. Based on this, we focused on the epigenetic mechanisms controlling the selective expression of TEAD4 isoforms. DNA methylation analysis (bisulfite sequencing) was performed on genomic DNA extracted from umbilical cord and placental samples. The two TEAD4 promoters, canonical and alternative, showed different DNA methylation profiles. Our bisulfite sequencing results were consistent with the protein expression patterns we observed. In both umbilical cord and placenta samples, the canonical TEAD4 promoter did not show methylation, which correlates with TEAD4 protein expression. Conversely, the truncated TEAD4-ΔN isoform promoter showed a significant methylation abnormality in umbilical cord samples, leading to transcriptional suppression and consequently to a lack of TEAD4-ΔN isoform expression in these samples.

4. DISCUSSION & CONCLUSIONS

The discovery of a new TEAD4 isoform, transcribed from an intronic promoter region, represents a major advance in our understanding of gene regulation. This isoform, TEAD4-ΔN, is expressed as a truncated variant at the N-terminal of the widely studied TEAD4 transcription factor. Interestingly, the TEAD4-ΔN transcript lacks the coding region for the traditional DNA-binding domain (TEA/ATTS), which makes this isoform a classic transcription factor. It is also supported by observations on the TEAD4-ΔN:RFP chimeric protein, which, lacking the nuclear localization signal (NLS), localizes predominantly in the cytoplasm of transfected cells, suggesting a non-nuclear function. Previous studies have described a shorter TEAD4 isoform that has been implicated as an alternative splicing product. Our experiments targeting the alternative splicing origin of TEAD4-ΔN were unsuccessful, despite attempts to detect it in a number of cell types. Our results demonstrate that alternative promoter usage, a more common event than alternative splicing, also leads to the production of this isoform, challenging the notion that its appearance is strictly related to tumorigenesis. The molecular and cellular function of TEAD4-ΔN is largely speculative. Remarkably, TEAD4-ΔN retains the full-length YAP-binding domain, raising the possibility of interacting with the YAP1 protein in the cytoplasm and influencing cell signalling mechanisms. Phosphorylation and nuclear translocation of YAP1, a key aspect of the Hippo signaling pathway, are critical processes in the regulation of cell proliferation and organ size. Therefore, understanding how TEAD4-ΔN influences the Hippo pathway, in particular its regulation and differentiation processes crucial for embryo implantation and placental development, may provide insights into the complex mechanisms that control these crucial developmental stages.

Our experiments show that TEAD4-ΔN mRNA expression is closely linked to the DNA methylation status of its intronic promoter. The lack of methylation in this region may promote a more open chromatin configuration, enhancing nucleosome decondensation and facilitating the binding of various transcription factors, including those with DNA-binding domains. Such an environment may enhance the gene regulatory role of TEAD4, as it may form heterodimers with other TEAD family proteins and SMAD transcription factors, further affecting gene expression. The role of DNA methylation in epigenetic regulation, particularly during critical preimplantation

and postimplantation periods of embryonic development, is well known DNMT3B, a key enzyme in de novo DNA methylation, is essential for modulating placental development and function. Thus, the methylation-dependent regulation of TEAD4-ΔN opens new avenues of investigation to explore the functional consequences of this isoform, particularly in the context of placental development and embryonic growth. The conventional form of TEAD4 without a DNA-binding domain is known to disrupt the Hippo-YAP signaling pathway, affecting cell proliferation, migration and organ growth. However, the specific molecular function of TEAD4-ΔN, including its interaction with other proteins and its role in various signaling pathways, is not yet fully understood. The elucidation of this mechanism could have important implications for therapeutic interventions and diagnostics. For example, manipulation of TEAD4-ΔN expression may offer new strategies for cancer therapy, potentially disrupting malignancy-related signaling pathways. Furthermore, TEAD4-ΔN may serve as a biomarker for certain cancers, providing diagnostic and prognostic value.



DNA methylation and its effect on the expression of TEAD4 isoforms. The A promoter directs the expression of full-length TEAD4 (TEAD4-FL), remaining unmethylated in various tissues, ensuring the expression of this isoform. In contrast, promoter B, which is responsible for the expression of the truncated isoform (TEAD4-ΔN), exhibits tissue-specific methylation, resulting in differential expression of this isoform in different tissues. The graphical representation shows CpG dinucleotides represented as "lollipops"; open lollipops indicate unmethylated CpGs, while those filled in black indicate methylated CpGs.

5. PUBLICATIONS

Peer-Reviewed Papers:

The paper is directly associated with the PhD thesis.

Rashidiani S, Mamo G, Farkas B, Szabadi A, Farkas B, Uszkai V, Császár A, Brandt B, Kovács K, Pap M, Rauch TA. Integrative epigenetic and molecular analysis reveals a novel promoter for a new isoform of the transcription factor TEAD4. Int J Mol Sci. 2024 Feb 13;25(4):2223. doi: 10.3390/ijms25042223.PMID:38396900.

IF: 5.6

További publikációk:

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IF: 0.8

Konferenciák:

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2. Rashidiani S, Pap M & Rauch TA. DNA methylation governed expression of the alternative TEAD4 promoter. Annual Meeting of the Hungarian Biochemical Society 2022. 25-27 August 2022. Pécs, Hungary.

3. Rashidiani S, Pap M & Rauch TA, Epigenetic profiling of the human chromosome 12 (p13.33) region reveals a novel promoter of the TEAD4 transcription factor. 29th International Student Congress Of (bio)Medical Sciences. 8-10 June 2022, Netherlands.

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