Published in final edited form as:

Neuromolecular Med. 2014 March ; 16(1): 16–24. doi:10.1007/s12017-013-8285-3.

Epigenetic Mechanisms of Rubinstein-Taybi Syndrome

Elizabeth Park^{1,#}, Yunha Kim^{3,#}, Hyun Ryu¹, Neil W. Kowall^{1,2}, Junghee Lee^{1,2}, and Hoon Ryu^{1,2,3,*}

¹Boston University Alzheimer's Disease Center and Department of Neurology, Boston University School of Medicine, Boston, MA 02118

²VA Boston Healthcare System, Boston, MA 02130

³Laboratory for Neuronal Gene Regulation and Epigenetics, Center for Neuro-Medicine, Brain Science Institute, Korea Institute of Science and Technology, Seoul 110-799, South Korea

Abstract

Rubinstein-Taybi Syndrome (RTS) is an incurable genetic disorder with combination of mental retardation and physical features including broad thumbs and toes, craniofacial abnormalities, and growth deficiency. While the autosomal dominant mode of transmission is limitedly known, the majority of cases are attributable to *de novo* mutations in RTS. The first identified gene associated with RTS is CREB-binding protein (*CREBBP/CBP*). Alterations of the epigenetic 'histone code' due to dysfunction of the CBP histone acetyltransferase (HAT) activity deregulates gene transcription that are prominently linked to RTS pathogenesis. In this review, we discuss how *CBP* mutation contributes to modifications of histone and how HDAC inhibitors are therapeutically applicable to epigenetic conditioning in RTS. Since most genetic mutations are irreversible and therapeutic approaches are limited, therapeutic targeting of reversible epigenetic components altered in RTS may be an ideal strategy. Expedient further study on the role of the epigenetic mechanisms in RTS is encouraged to identify novel epigenetic biological markers and therapeutic targets to treat RTS.

Keywords

RTS; histone acetylation; histone methylation; CBP; HDAC

1. INTRODUCTION

History/Epidemiology/Risk Factors

Rubinstein-Taybi Syndrome (RTS) was first reported by Rubinstein and Taybi in 1963 for its distinct combination of mental retardation and characteristic physical features including broad thumbs and toes, craniofacial abnormalities, growth deficiency, and increased risk for childhood cancers (Rubinstein and Taybi 1963).

RTS is one of the rarest congenital syndromes, affecting anywhere between 1 in 100,000 to 720,000 newborns. Limited documented reports have indicated autosomal dominant mode of transmission (Hennekam 2006). However, majority of cases are attributable to *de novo*

*Corresponding author: Hoon Ryu, Ph.D. Department of Neurology Boston University School of Medicine VA Boston Healthcare System Building 1A, Rm 109 Boston, MA 02130 Tel: 1-857-364-5910; Fax: 1-857-364-4540; hoonryu@bu.edu.

#These authors are equally contributed.

Conflict of interest

The authors declare that they have no conflict of interest.

mutations (Roelfsema and Peters 2007). The calculated risk of recurrence after having a child with RTS is very low but the recurrence risk between RTS patient is reportedly as high as 50% (Cantani and Gagliosi 1998), which mandates careful genetic counseling for sexually active patients, especially given that sexual development e.g. onset of puberty, menarche in RTS adolescents matches to those of normal individuals (Wiley et al. 2003).

One of the first genes identified in association with RTS was *CBP/CREBBP*, encoding CREB (cAMP-response element binding protein)-binding protein (Petrij et al. 1995). *CBP/CREBBP* holds wide and variable functions. Using HAT (histone acetyltransferase) activity, *CBP/CREBBP* regulates gene expression by acetylation of histones H3 and H4, de-condensing chromatin and allowing for transcription. *CBP/CREBBP* also recruits and interacts with transcriptional machinery such as RNA polymerase II (Pol II) complex, co-activators and repressors, through its various domains including zinc finger domains and CREB-binding domain (KIX domain) (Fig. 1b). *CBP/CREBBP* additionally regulates the expression of histone methyltransferase, leading to hyper-trimethylation and chromatin condensation, and effectively, gene silencing (Urduingio et al. 2009). Lastly, *CBP/CREBBP* acetylates p53, a tumor suppressor pathway known to be deregulated in many human cancers, among other proteins to ultimately activate target genes.

Heterozygous germ line mutations of the *CBP/CREBBP* located on chromosome 16p13.3 are associated with RTS majority of the time (50%). Up to this point, specific *CBP/CREBBP* mutations have been detected in 41% of patients (Caglayan et al. 2011; Couprie et al. 2004; Hennekam et al. 1993; Hennekam 2006). Unsurprisingly, *CBP/CREBBP* mutations are quite heterogeneous and 92 different mutations have been identified in the HAT (histone acetyltransferase) and KIX domains (consisting of 13 missense, 20 nonsense substitutions, 10 splicing substitutions, 16 small deletions, 2 small indels, 19 gross deletions, 9 small insertions, 1 gross insertion, and 2 complex rearrangements) by the Human Gene Mutation Database (www.hgmd.org) (Fig. 1a) (Bartholdi et al. 2007; Bartch et al. 1999; Blough et al. 2000; Couprie et al. 2002; Couprie et al. 2004; Demeer et al. 2013; Hou 2005; Wallerstein et al. 1997). A much smaller percentage of these mutations are due to *CBP/CREBBP* homologue EP300 (E1A binding protein p300) on chromosome 22q13.2, while the rest of the cases remain unaccounted for (Hallam and Bourtchouladze 2006). However, new mutations in *CBP* are being reported as recent as this year (Suzuki et al. 2013).

Given that molecular mutations of *CBP/CREBBP* and p300 only account for half of all of the observed phenotypic features in RTS, it is possible that other epigenetic mechanisms affecting histone acetylation and subsequently gene transcription also contribute to the development of RTS. Therefore, this review paper seeks to summarize currently known epidemiology, diagnosis, treatment, and epigenetic pathophysiology behind RTS and suggest a new mechanism involving p53, microRNAs and *CBP/CREBBP/p300*.

Clinical Features/Diagnostic Methods

Several classic clinical facial and limb characteristics are associated with RTS. Facial features include high arched eyebrows, down-slanting palpebral fissures, and broad nasal bridge. Special attention must be given to facial expression, as grimace, or an extraordinary smile with closing of the eyes is almost always observed. Most common limb abnormalities include broad thumbs and broad big toes. Partial duplication of digits, deviation of thumbs and halluces, terminal broadening of phalanges, and fingers may all be present. Growth delays during infancy followed by excessive weight gain in childhood are common, accompanied by global mental retardation and IQs ranging from <25 to 79 with cognitive delay (Balci et al. 2004; Beluffi et al. 1987; Kumar et al. 2012)

Special attention is also mandated for internal organ anomalies including heart malformations, such as PDA (patent ductus arteriosus) or atrial/ventricular septal defects, kidney abnormalities, and hypospadias (male urethra birth defect involving an abnormally placed urinary opening on the underside of the penis). Secondary risk seizures may occur, the mechanism of which can be postulated from the fact that changes in the state of chromatin can affect the expression of specific genes involved in the seizure, as demonstrated in epilepsy (Urduingio et al. 2009). Screening and maintenance should be carried out according to current medical guidelines. For instance, regardless of the age at diagnosis, an evaluation by a pediatric geneticist knowledgeable in dysmorphology and development, an ECG (electrocardiogram) and an echocardiogram and examination by pediatric cardiologist, a full ophthalmologic exam by a pediatric ophthalmologist, a renal ultrasound and possible VCUG (voiding cystourethrogram) and hearing evaluation, are all required as baseline studies (Wiley et al. 2003). Notably, RTS patients are predisposed to tumors of the brain, meningioma, leukemia, as well as tumors of neural or developmental origins (neuroblastoma, medulloblastoma, rhabdomyosarcoma...etc) but with normal life expectancy (Miller and Rubinstein 1995). While no official diagnostic criteria for RTS exists, examining for key clinical features through meticulous history taking and physical exam (head & neck, dental inspection, skin exam for keloids, radiographs of hands, height, and growth chart) may initiate the diagnostic process (Giussani et al. 2012; Harth and Linse 2001; Labenne et al. 1990; O'Neil et al. 1989). After establishing clinical diagnosis, chromosome analysis using FISH for probe detection of microdeletions at chromosome 16p13.3 or if no microdeletions are established, molecular analysis for mutations in CBP/CREBBP and p300 should follow (Hennekam 2006; Tsai et al. 2011; Udaka et al. 2006).

2. HOW ARE EPIGENETIC ALTERATIONS MODULATED BY CBP?

Heterogeneous mutations in CBP/CREBBP domains and subsequent epigenetic alterations manifest as cognitive deficits, development of cancers, and numerous phenotypic features characteristic of RTS (Fig. 1b). Even a small disruption of the PHD (Plant Homeo Domain) finger mutation (E1278K) or deletion of exon 22, encoding a central region of PHD finger is sufficient enough to cause loss of CBP acetyltransferase activity and lead to RTS (Kalkhoven et al. 2003). The PHD region is critical for acetylation of histones, CBP/CREBBP autoacetylation, and contains the basal transcription factor TFIIE34 (Kalkhoven et al. 2002).

Multiple models are possible given the tremendous variation in phenotype and in mental retardation. Two models on the forefront include: haploinsufficiency and dominant negative inhibition (Hallam and Bourtchouladze 2006). Both models are supported by the observations that not just null mutants but also truncated mutants can cause RTS in humans (implying that 2 functional copies are necessary to produce sufficient protein) and that injection of a CBP-CREB binding domain (KIX domain) into fibroblasts blocks transcriptional activation of CRE-lacZ reporter gene (signifying that the abnormal product derived from mutant allele inhibits the wild type product) (Hallam et al. 2006). CBP/CREBBP is known to bind with partners affecting cellular patterns and during almost every stage of development and embryogenesis but a specific link to mental retardation has yet to be established.

CBP/CREBBP mutations leading to cognition changes may be due to the fact that CBP/CREBBP is activated by various signaling pathways (PKA, MAPK, and CaMKIV) in physiological processes like learning and long-term memory formation (Abel et al. 1997; Atkins et al. 1998; Bito et al. 1996). Mouse deletion models offer some lead on such a link (Oike et al. 1999). Oike et al (Oike et al. 1999) first demonstrated that homozygous CBP/CREBBP mice mutants were embryonic lethal but heterozygous mutant mice are less viable

and show growth retardation with defective long-term memory. Hallam et al. 2006 used heterozygous CBP/CREBBP mutant mice to observe whether long-term memory formation could be improved via enhancement of upstream signaling onto CBP and CREB by using PDE4 inhibitors. Delivery of PDE4 (phosphodiesterase-4) inhibitors before training restored long term memory in heterozygous mutant mice to normal levels (Hallam and Bourtschouladze 2006). This could be explained by the increase of the magnitude or the duration of cAMP levels via blocking the degradative action of PDE4 on cAMP, which would ultimately increase functional activation of CBP and CREB molecules and manifest downstream during memory formation.

Tanaka et al. (1997) generated a null allele mouse model of RTS. Interestingly, only a few of the mutant mice (7 of 21) showed the same phenotypic features expected of RTS. Further testing by Alarcon et al (Alarcon et al. 2004). in those heterozygote mice showed deficient long-term memory and decreased LTP (long-term potentiation) which were corrected by PDE4 inhibitors or overexpression of CREB. Likewise, reduced HAT activity in these mutant mice could also be treated with HDAC (Histone Deacetylase inhibitor SAHA), reversing memory deficit (Tanaka et al. 1997). Two other groups (Korzus et al. 2004; Wood et al. 2005) actually localized the CBP/CREBBP gene to the forebrain, further supporting CBP/CREBBP's role in long-term memory (Haettig et al. 2011; Kim et al. 2012; Korzus et al. 2004; Stefanko et al. 2009; Wood et al. 2005).

Our own data expands on the role of CBP/CREBBP in neuron function via a new mechanism. We discovered that monoallelic deletion of CBP/CREBBP leads to the induction of ESET/SETDB1 (ERG associated protein with SET domain), a histone-specific trimethyltransferase, which increases the trimethylation of histone H3 Lys 9 (H3K9me3) and ultimately leads to condensation of pericentromeric heterochromatin structure in neurons and striatal neuron atrophy and dysfunction (Lee et al. 2008) (Fig. 2). Moreover, in heterozygote mice (CBP^{+/-}), Ets-2 (erythroblastosis virus E26 oncogene homolog 2) transcription factor regulating transcriptional activity of the ESET gene had increased occupancy in the promoter DNA of ESET. We therefore postulated that CBP/CREBBP is transcriptional repressor of ESET gene via limitation of Ets-2 transcriptional activity, while CBP/CREBBP siRNA enhances Ets-2 dependent ESET transcriptional activity (Lee et al. 2008). In turn, ESET increases the level of H3K9me3. Heterochromatin condensation induced by H3K9me3 represses the expression of *CHRM1* gene and leads to the subsequent reduction of CHRM1 protein in neurons. The decreased level of CHRM1 fails to respond to acetylcholine (ACh) from cholinergic interneurons and to transduce G-protein-coupled intracellular Ca²⁺-dependent signaling pathway. Consequently, the deregulation of CHRM1-dependent synaptic function may contribute to behavioral abnormality (Fig. 2) (Lee et al., 2013).

The interaction between CBP/CREBBP, p300, and p53 is also well researched. p53 (tumor suppressor pathway) is known to be dysregulated in most human cancers. p53 activity is controlled by various post-transcriptional methods, including cytoplasmic mono- and poly-ubiquitination for ligase driven (MDM2 (Mouse double minute 2 homolog), catalyzing addition of single ubiquitin moieties to p53) proteasome targeting. Specific interactions between p300, CBP/CREBBP, acting as co-activators, and MDM2, play a key role in p53 turnover (Grossman et al. 1998). p300/CBP modulate the p53 pathway at multiple levels (Grossman 2001). p300 contributes to maintaining p53 stability by regulating its ubiquitination and degradation, through both MDM2-dependent and -independent mechanisms (Grossman et al. 2003; Kawai et al. 2001). After DNA damage, p53 is activated by phosphorylation by various kinases and acetylation at specific amino-acid residues by p300/CBP (Sakaguchi et al. 1998). P53 acetylation is thought to promote target gene activation by increasing the stability of the p53–p300–DNA complex (Gu and Roeder 1997).

Furthermore, p300 is required for p53-mediated transactivation of target genes through its co-activator function and acetylation of target gene histones (Grossman et al. 1998). Therefore, loss of CBP/p300 could disrupt p53 activation, stability, and transactivation of target genes and alter the cell fate in response to p53 activation. In fact, p300 knockdown by hammerhead ribozymes inhibits apoptosis, probably by disrupting the p53-mediated response to DNA damage (Yuan et al. 1999a; Yuan et al. 1999b).

As discussed above, CBP/p300 regulate p53 activation via acetylation. Given that CBP and p300 are known to be dysregulated in RTS and form critical interactions with MDM2/CREBBP for p53 regulation, questions are raised regarding the role that p53 may play in RTS itself and its relation to RTS predisposition to certain types of cancers. Of note, some somatic CBP/CREBBP and p300 mutations in sporadic tumors have been reported, but to a lesser degree than p53. CBP/CREBBP translocations are common in AML (Acute Myelogenous Leukemia) and truncation of p300 protein products or amino acid substitutions in critical protein domains with inactivation of the second allele have been observed in solid tumors. However, to date, no syndrome involving the germline mutation of the p300 gene has been described (Iyer et al. 2004). Those rare tumors of neural crest and developmental origin, including neuroblastoma, medulloblastoma, meningioma, and pheochromocytoma developed by pediatric RTS patients have been difficult to obtain for analysis and therefore unable to demonstrate somatic inactivation. Because of the established relationship between CBP/p300 and p53, the prevalence of specific tumors in RTS, the role that small non-coding RNAs may play in interacting with CBP/CREBBP and subsequent effects on heterochromatin is worth considering.

Generally, disruption of the RNA interference (RNAi) pathway is hypothesized to lead to relaxation of heterochromatin around centromeres, which may induce expression of normally unexpressed genes and subsequent decrease in histone H3 methylation. Conversely, gene silencing by posttranscriptional (siRNA, microRNA) and transcriptional (antisense RNA) small noncoding RNA has been demonstrated *in vivo* in tumor suppressor genes like p53 or genes associated with proliferation/apoptosis (Wnt signaling, Bcl2 family), resulting in cancerous proliferation of cells leading to tumor formation (Devaskar and Raychaudhuri 2007). Small noncoding double stranded RNAs (dsRNAs) are also specifically observed in neural progenitors of adult hippocampus. Expression of dsRNA converts NRSF (neuron-restrictive silencing factor, also called REST) from repressor to activator complex, leading to neuron-specific gene activation and neurogenesis (Kuwabara et al. 2005).

More specifically, microRNAs (miRNAs) are involved in cellular development, differentiation, and apoptosis. These molecules consist of 19 to 24 bp double stranded RNAs which mediate gene silencing and consequently a great number of biological processes, including cell proliferation, developmental timing, fat metabolism, and hematopoietic/adipocyte differentiation (Ghosh et al. 2012; Mizuguchi et al. 2012; Servillo et al. 2002; Vo et al. 2005). So far, there is no direct evidence that CBP/CREBBP is involved in miRNA metabolism. CREB, a CBP interacting transcription factor, is known to regulate the expression of miR132, a miRNA enriched in neurons and induced by neutrophins, identified through genome wide screening. Expression of miR132 in cortical neurons induces neurite outgrowth while inhibition attenuated outgrowth, emphasizing the importance of the role of CREB in neuronal morphogenesis. (Vo et al. 2005). In addition, miR-372, when overexpressed, decreases CREB's association with proximal promoter, followed by dissociation of CBP/p300, changing histone deacetylation/methylation (Wang et al. 2010). Therefore, more elucidation of the mechanism behind CBP/CREBBP-dependent miRNA metabolism remains to be determined in future study.

3. CONCLUSION & FUTURE PERSPECTIVES

Clinical Management/Therapy

Proper genetic counseling while keeping in mind that recurrence risk is extremely low should be given with prenatal diagnosis, when appropriate. Vigilance in the first year of life is key, particularly to feeding problems, congenital cardiac defects, glaucoma, and airway issues. As patients progress, delayed motor and cognitive development and behavioral problems should also be continuously addressed. Patients will enter schools for students with learning disabilities. Special attention should be paid for evolving behavioral problems into early adulthood including short attention span, stubbornness, lack of persistence, and sudden mood changes (Hennekam 2006; Verhoeven et al. 2010; Yagihashi et al. 2012).

Treatment options have been somewhat limited. The one option exercised so far has been HDAC (histone deacetylase) inhibitors. Histone acetylation is regulated through the concerted activities of HAT (histone acetylases), which acetylates lysine residues in histone tails, transforming nucleosomal structure and ultimately increasing DNA transcription, and HDACs (Histone deacetylases), which removes those acetyl groups from lysine/arginine residues and subsequently inhibits transcription (Bannister et al. 2002; Marks et al. 2000). HDAC inhibitors can promote transcription activation or suppression by relaxing DNA conformations in a gene context-dependent manner. HDAC inhibitors have been tested preclinically in various neurodegenerative conditions with perturbations of histone homeostasis such as animal models of HD (Huntington's Disease), ALS (Amyotrophic Lateral Sclerosis), and Parkinson's Disease (Alarcon et al. 2004; Camelo et al. 2005; Ferrante et al. 2004; Gardian et al. 2005; Ryu et al. 2006; Sugai et al. 2004). As mentioned previously, mice with genetic alterations of CBP/CREBBP (single null mutation with truncated form of CBP or dominant negative form of CBP) have deficits in LTM (long term memory) (Hahnen et al. 2008). Hippocampal L-LTP (long-term potentiation) was subsequently improved by use of HDAC inhibitors (Kazantsev and Thompson 2008). Moreover, treatment of RTS utilizing inhibitors such as SAHA (Suberoylanilide Hydroxamic Acid) or TSA (Trichostatin A) improved deficits in synaptic plasticity and cognition in several CBP/CREBBP mutant lines of mice (Abel and Zukin 2008; Bourtschouladze et al. 2003; Korzus et al. 2004; Vecsey et al. 2007). However, testing of these HDAC inhibitors in human RTS has yet taken place (Fig. 3).

Alternative methods to target this loss of CBP/CREBBP are worth considering. CBP/CREBBP loss of function leads to a decrease in histone acetylation levels along with a decrease in CBP/CREBBP-dependent transcription. General HDAC inhibition would reverse CBP-dependent decrease in acetylation but also increase acetylation levels at non-specific promoters across wide variety of genes while CBP/CREBBP activation (by overexpression or pharmacologic method) would only specifically target CBP/CREBBP-dependent histone acetylation and transcription (Fig. 3) (Rouaux et al. 2003). Given that CBP/CREBBP overexpression could reverse neuronal apoptosis and that neuroprotective effects of CBP over-expression are dependent on HAT activity (Rouaux et al. 2003), focusing on CBP/CREBBP activators as neuroprotective drugs and development of more selective HDAC inhibitors would be beneficial (Balasubramanyam et al. 2003). In the realm of inherited genetic disorders, most genetic mutations are irreversible and therapeutic approaches are limited. However, epigenetic modifications are considered to be highly reversible phenomenon. Accordingly, therapeutic targeting and modulation of epigenetic components altered in RTS might be an ideal method and promising future treatment modality.

Acknowledgments

This study was supported by NIH NS 067283 (H.R.) and Brain Science Flagship Grant (2E24380) (H.R.) from Korea Institute of Science and Technology (KIST).

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Fig. 1.

Microdeletions and mutations of *CREBBP* (*CBP*) gene can cause Rubinstein-Taybi syndrome (RTS). **a** scheme illustrates mutations of KIX domain, plant homeodomain (PHD)-type zinc finger domain, and histone acetyltransferase (HAT) domain in CBP protein that are found in patients with RTS. CH1, the first cysteine/histidine rich region (also known as TAZ1); KIX, CREB and MYB interaction domain, BD, bromo domain; CH3, the third cysteine/histidine rich region (also known as TAZ2); p160, p160 binding site; TAD, transactivation domain. **b** CBP dysfunction due to its mutations leads to epigenetic modification. CBP harbors intrinsic HAT domain and provides functional HAT activity. Mutations and deletions of CBP HAT domain decrease acetylation of histones, close the chromatin structure, and impair gene expression. In addition, CBP is a transcriptional coactivator. The mutation of KIX domain can prevent interaction of CBP with the cAMP response element (CRE) binding protein (CREB) and other DNA-binding transcription factors and consequently deregulates initiation of gene transcription through the RNA polymerase II complex. In addition, deregulation of CBP function decreases acetylation of UBF and UBF-mediated ribosomal DNA (rDNA) transcription.

Fig. 2.

CBP deficiency leads to HMT-mediated epigenetic modification. Induction of HMT such as ESET/SETDB1, a H3K9-specific methyltransferase, by CBP deficiency via a nonconventional pathway can elevate the level of H3K9me3, a histone code for gene silencing, and increase the condensation of heterochromatin. The alteration of gene transcription ultimately contributes to neuronal dysfunction. For example, H3K9me3-induced heterochromatin condensation results in the repression of *CHRM1* gene and subsequent reduction of *CHRM1* protein in neurons. The reduction of *CHRM1* fails to respond to acetylcholine (ACh) from cholinergic interneurons and to transduce G-protein-coupled intracellular Ca^{2+} -dependent signaling pathway. As a result, deregulation of *CHRM1*-dependent synaptic function may contribute to behavioral abnormality (modified from Lee et al., 2013).

Fig. 3.

The rescue of epigenetic alteration by HDAC inhibitors may be an ideal therapeutic strategy to improve symptoms of RTS. Since the level of acetylated histone, a representative histone code for transcriptional activation, is decreased due to the dysfunction of CBP HAT activity in RTS, HDAC inhibitors can restore the level of acetylated histone by inhibiting the activity of histone deacetylase and ameliorate the symptoms of RTS. In this regard, expeditious studies on the therapeutic modulation of reversible epigenetic modification and gene transcription in cellular and animal models of RTS are necessary.