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REVIEW

Indications to Epigenetic Dysfunction in the Pathogenesis of Common Variable Immunodeficiency

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Abstract Primary immunodeficiencies (PIDs) are a group of rare genetic diseases resulting in the impairment of one or more functions of the human immune system. Common variable immunodeficiency (CVID) is one of the most prevalent PIDs, yet despite extensive genetic analysis, most patients do not have a monogenetic diagnosis. This has led to the theory that CVID must be a polygenetic condition. An alternative theory to a monogenetic or polygenetic underlying cause of CVID is that it is epigenetic phenomena that are causal in the majority of CVID patients. I will briefly discuss epigenetic regulation in B-cell biology and development, current examples of epigenetic diseases causing CVID-like primary antibody deficiencies, and how these observations may guide future investigation into the role of epigenetics in CVID.

Keywords Epigenetics · B-cell development · Common variable immunodeficiency

Introduction

Primary immunodeficiencies (PIDs) are a group of rare genetic diseases resulting in the impairment of one or more functions of the human immune system, and nearly 300 monogenic PIDs have now been described (Picard et al. 2015). Within this group, there is a large variation in both the clinical phenotype between genetically different PIDs and in cohorts of PID patients with the same genetic variant. Treatment for PID is directed by: clinical assessment, immunological phenotype, and the severity of the disease. Increasing importance is being now placed on the genetic diagnosis in PID, as it is being used to guide prognosis and earlier treatment choices. An example of this is the newborn screening program that facilitates early, often pre-symptomatic, diagnosis in neonates with severe combined immunodeficiency (Buelow et al. 2014; Gaspar et al. 2014). The molecular abnormalities in PID are now also guiding precision treatments, further emphasising the importance of a genetic diagnosis (Zhang et al. 2015b). Common variable immunodeficiency (CVID) is one of the most prevalent PIDs. As implied in name, CVID is a highly heterogeneous and variable clinical condition with broad diagnostic criteria. The diagnostic criteria of the European Society for Immunodeficiencies 2014 require several clinical and immunological parameters (Ameratunga et al. 2014) (Supplementary Table 1). This clinical heterogeneity in CVID has led to diagnostic challenges and difficulties in determining the optimal treatments for patients (Gathmann et al. 2014).

Diagnostic Challenges in CVID

Despite extensive genetic analysis, most patients with a diagnosis of CVID currently do not have a monogenetic molecular diagnosis. The majority of CVID cases do not follow a classical Mendelian pattern of inheritance, often with single sporadic cases in families. The age of diagnosis in CVID cases demonstrates a bimodal distribution of 5–10 years of age and 30–40 years of age, which is at odds with other monogenic PIDs that usually present in the

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neonatal period or during early infancy (Gathmann et al. 2014). Studies of the genetics of CVID cohorts typically identify a Mendelian monogenetic diagnosis in only approximately 5–10 % of cases (van Schouwenburg et al. 2015). Genome-wide association studies of CVID patients have shown associated single nucleotide polymorphisms or copy number variations, but with insufficient power to infer causality (Keller et al. 2014; Li et al. 2015a; Orange et al. 2011). This lack of monogenetic diagnoses has led to the theory that CVID must be a polygenetic condition in the majority of cases (Orange et al. 2011; van Schouwenburg et al. 2015). An alternative theory to a monogenetic or polygenetic underlying cause of CVID is that it is epigenetic phenomena that are causal in the majority of currently molecularly undiagnosed CVID patients.

Epigenetics-Regulation of Gene and Protein Expression

Epigenetics is an integrated, dynamic, and potentially reversible process that causes changes in gene expression without altering the germ-line DNA gene sequences. These changes can accumulate over the life span of an organism and are influenced by many factors, including environmental, infectious, nutritional, and iatrogenic stimuli that alter the epigenome of an individual. Several epigenetic mechanisms account for the alterations of gene expression and are a vital part of normal development and specialized cell differentiation. These epigenetic mechanisms include cell-specific transcription factor expression, DNA methylation, histone and chromatin modification, and non-coding RNAs (ncRNAs) (Alberghini et al. 2015; Elia and Condorelli 2015) (Fig. 1).

Transcription Factors

Expression of genes relies on binding of transcription factors to gene-specific promoter and enhancer DNA regions (Levine and Tjian 2003). Transcription factor binding to DNA recruits RNA polymerase II leading to the formation of the transcription initiator complex and gene transcription. The differential expression of specific transcription factors within specialized cell subtypes (e.g. Foxp3 in regulatory T cells) in one of the key epigenetic methods for determining the subsequent cell function and genes that are transcribed within the cell.

DNA Methylation

5′—cytosine—phosphate—guanine—3′ motifs (CpG) are sequences of DNA that are usually occur in clusters (known as CpG islands) located 5′ upstream of gene promoters within the mammalian genome. CpG methylation results in gene silencing by reducing transcription factor binding and altering chromatin structure to render DNA sequences inaccessible to transcription machinery (Fig. 1). DNA methyltransferase enzymes (DNMTs) are one of the most important class of enzymes involved in DNA methylation and maintenance of genome methylation. Abnormalities in DNA methylation status can have severe impacts on normal growth, development, and cellular processes (Robertson 2005). Within the immune system, fluctuation in methylation is important in cellular functions, for example, the need for DNA site specific demethylation to occur in Natural Killer (NK) cells to allow NK effector functions to become active (Wiencke et al. 2016).

Histone/Chromatin Modification

Histones are alkaline proteins around which DNA is wound to form chromatin. Modifications to histone proteins that reduce their positive charge reduce the force of attraction with negatively charged DNA. This has the effect of ‘loosening’ the winding of the DNA around the histone and opening up of chromatin (to euchromatin) allowing transcription factors to access DNA-binding regions (Fig. 1). Enzymes that cause histone modifications can be grouped as either enhancers of chromatin variegation (E(var)s) resulting in opening of chromatin and increasing gene transcription, or suppressors of chromatin variegation (Su(var)s)—histone deacetylase, histone methylases, and protein phosphatases, which cause heterochromatin compaction and reduction of gene transcription by causing inaccessibility of DNA transcription factor-binding sites.

Non-Coding RNAs

NcRNAs are RNA molecules transcribed from DNA that are not translated into proteins, but instead exert regulatory effects on protein translation by influencing DNA transcription and mRNA post-transcriptional changes. ncRNAs can be subdivided into long or short ncRNAs. Micro RNAs (miRNAs) make up an important subgroup of short RNAs that are involved in many epigenetic regulatory processes and function mainly to repress gene expression within a cell (Elia and Condorelli 2015). MiRNAs have a wide range of significant functions across the immune system, including an important role in peripheral B-cell maintenance and function. In the peripheral lymphoid compartment, B-cell survival is influenced by differential miRNA expression exerting epigenetic control on gene expression in B cells as they mature from a naïve state

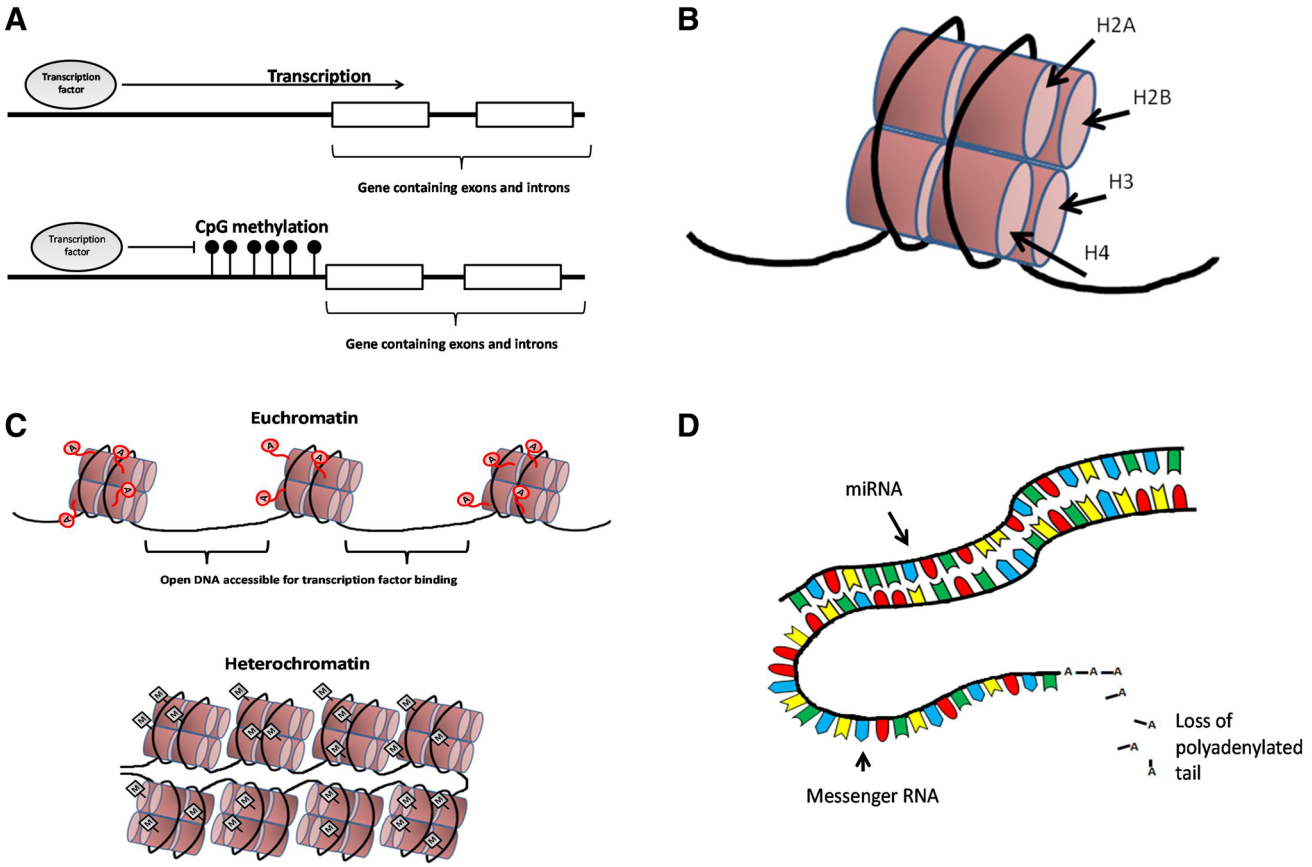

Fig. 1 **a** Methylation of CpG islands, often located 5′ upstream of the gene promoter region, reduces transcription of the downstream gene by inhibiting transcription factor binding to gene promoter regions. **b** Nucleosomes contain 8 histone proteins (2 copies of H2A, H2B, H3, H4) with DNA wound around them. The acetylation and methylation of arginine and lysine residues on histone proteins alter their charge and the strength of DNA binding, contributing to chromatin remodelling, DNA unwinding, and active gene transcription. **c** In general, DNA and histone demethylation and histone acetylation alter chromatin structure to euchromatin and allow DNA to be accessible to transcription factors. DNA and histone methylation conversely condense chromatin structure to heterochromatin and make DNA inaccessible to transcription factors. **d** MicroRNAs (miRNA) are short non-coding RNAs that repress messenger RNA (mRNA) translation by binding to 3′ untranslated regions in mRNA causing mRNA degradation, and can also cause deadenylation of mRNA poly-A tail within the cytoplasm. Some miRNAs can also bind DNA and influence DNA methylation and histone modification (Elia and Condorelli 2015)

through to terminal effector cells (Bao and Cao 2016; Basso et al. 2009).

Epigenetic Regulation of the Immune System in Health and Disease

Deleterious epigenetic changes that may occur throughout life have been shown to result in diseases, including malignant haematological diseases and autoimmune conditions (Ansell 2015; Jeffries and Sawalha 2015). The immune system is not protected from epigenetic changes; in fact, it is potentially more susceptible to detrimental epigenetic changes due to the need for rapid, frequent functional and morphological changes that individual immune cells are required to undergo during adaptive immunological cell development and responses (Knight 2013). Exceptionally rare cases of monozygotic twins with PIDs and discordant clinical phenotypes have hinted at the complex role of epigenetic regulation in the immune system (Buchbinder et al. 2011; Rodríguez-Cortez et al. 2015).

Normal B-cell development is subject to multiple complex epigenetic regulations via: differential transcription factor expression at developmental time-points, DNA methylation status fluctuations, histone modifications, and ncRNA actions (Bao and Cao 2016). Similar to acquired epigenetic mutations identified in haematological malignancies (Ansell 2015; Jiang and Melnick 2015), could epigenetic abnormalities effect B-cell function and result in CVID? (van de Ven et al. 2011)

Herein, I will briefly discuss the background of epigenetic regulation in B-cell biology and development, current examples of epigenetic diseases causing CVID-like primary antibody deficiencies and how these observations

may guide future investigation into the epigenetic causes of CVID.

Epigenetics of Early B-Cell Development

Early haematopoietic stem cell (HSC) differentiation to common lymphoid progenitor cells is reliant on transcription factor expression (e.g. PU.1, Ikaros), ncRNAs, and also DNA methylation status (Bao and Cao 2016) (Fig. 2). Methylation of CpG rich islands, by DNMTs, reduces the associated gene expression and influences many developmental processes in the human body. HSC differentiation is one process sensitive to DNA methylation changes. DNA CpG methylation status dictates specific gene expression within HSCs, and DNA hypomethylation promotes myeloid lineage commitment as opposed to lymphoid (Bird 2002; Bröske et al. 2009). The differential expression of many transcription factors, including E2A, EBF1, Ikaros, and PAX5 is epigenetically regulated in B cells at various time-points by chromatin remodelling and DNA methylation (Lin et al. 2010; Reynaud et al. 2008). The correct sequential expression of different transcription factors during B-cell maturation is, therefore, closely controlled by the epigenetic regulation.

The development of pro-B cells requires expression of the pre-B-cell receptor (BCR). Pre-BCR expression is achieved by translation and expression of the immunoglobulin heavy chain (μ-H-chain) with the surrogate light chain V-pre-B lambda 5 (Pieper et al. 2013). The process of $V_HD_HJ_H$ recombination of the pre-BCR requires the recombinase activating gene proteins (RAG1 and RAG2) accessing and binding immunoglobulin heavy chain recombination signal sequences (RSS) on DNA (Stanhope-Baker et al. 1996). In 1996, it was reported from in vitro studies that the rearrangement of T- and B-cell receptor DNA regions was dependent on chromatin structure, allowing access of RAG1 and RAG2 to these particular RSS' (Stanhope-Baker et al. 1996). This is an early example of importance of epigenetics in B-cell development.

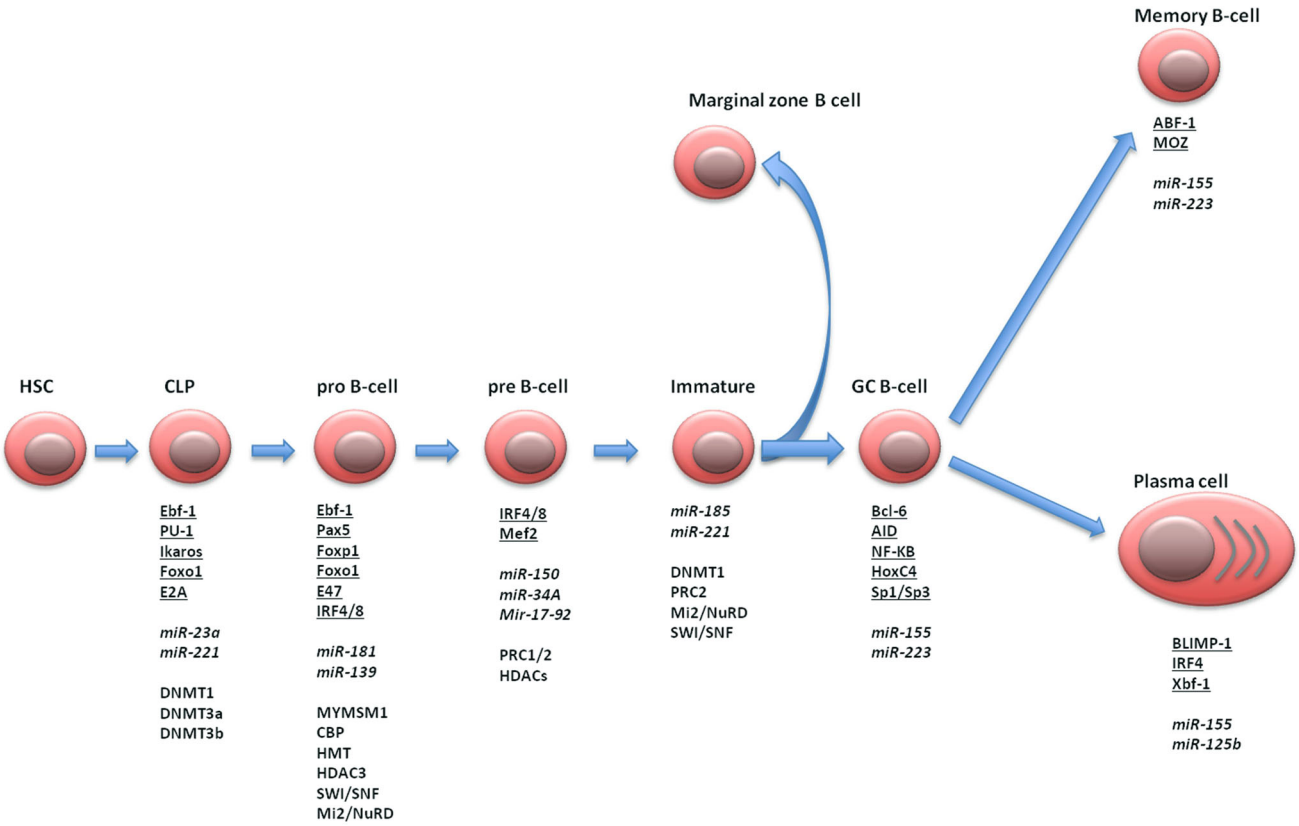

Fig. 2 Diagram of some defined epigenetic factors influencing B-cell development. More is known about the epigenetic control of the sequential development of B cells within the bone marrow, then the complex interactions and B-cell subgroups within the periphery (Bao and Cao 2016; Chiu et al. 2014; Herglotz et al. 2016; Zan and Casali 2015). Different B-cell subtypes with differing epigenetic profiles are present within the germinal centre. *Underlined* text: transcription factors, *italics*: *miRNAs,* and text: histone modification factors. *Evidence of a variants causing human immunodeficiency; Ikaros (Goldman et al. 2012; Kuehn et al. 2016), DNMT3b (Weemaes et al. 2013), IRF4 (Indrevær et al. 2015), AID (Revy et al. 2000), NF-KB (Chen et al. 2013; Fliegauf et al. 2015), KMT2D (Lin et al. 2015), KDM6A (Miyake et al. 2013). +Evidence of a mutation causing CVID-like disease in mice only; *miR-17-92* (Xu et al. 2015), *miR-142* (Kramer et al. 2015), *miR-155* (Rodriguez et al. 2007), and *miR-210* (Mok et al. 2013)

Methylation Disorder: Immunodeficiency with Centromeric Instability and Facial Anomalies

The PID condition, immunodeficiency with centromeric instability and facial anomalies (ICF) 1, is caused by a genetic mutation and resultant loss-of-function in the gene *DNMT3B* encoding the enzyme DNMT3b. DNMT3b is one of the methyltransferases active in HSCs in methylating DNA and promoting lymphoid cell differentiation. ICF1 shares many similarities with CVID displaying hypogammaglobulinaemia, decreased proportion of circulating memory B cells, and low levels of DNA CpG methylation (Weemaes et al. 2013). However, as expected, many ICF patients have a broader immunodeficient phenotype with T-cell impairment and lower total levels of lymphocytes. The striking immunodeficient condition of patients with ICF1 demonstrates the importance of correct methylation status during lymphocyte maturation and effector functions.

Histone/Chromatin Disorder: Kabuki Syndrome

Kabuki syndrome is a rare multi-system developmental delay disorder that often occurs due to mutation in one of the genes *KMT2D* or *KDM6A*, which encodes the histone and chromatin modification enzymes lysine methyltransferase 2D and lysine demethylase 6A, respectively (Miyake et al. 2013; Ng et al. 2010). These enzymes are involved in epigenetic regulation via their actions on histones and subsequent abilities of transcription factors to bind DNA promoter regions. Many Kabuki patients have immunological phenotypes similar to that of CVID with hypogammaglobulinaemia, poor vaccine responses, and a loss of class switched memory B cells and/or severely reduced naive B cells (Zhang et al. 2015a). These impaired B-cell functions are a result of incorrect histone epigenetic regulation that is required for B-cell activation and effector functions (Lin et al. 2015; Lindsley et al. 2016).

CD19 Transcription Defects: a Possible Dysfunctional Epigenetic Mechanism in CVID

All genes within the human genome are under epigenetic regulation. Due to aberrant expression of CD19 in haematological malignancies, the epigenetic regulation of *CD19* has been studied in great detail. Biallelic *CD19* gene variants have been described as a rare monogenetic cause of a CVID phenotype (van Zelm et al. 2006). With the knowledge of the epigenetics of *CD19* regulation, and the knowledge that *CD19* is a candidate gene for a CVID-like phenotype, we may speculate on an example of epigenetic *CD19* dysfunction as a factor in CVID pathogenesis.

CD19 is a critical co-stimulatory B-cell surface molecule during the activation of the BCR complex (Carter and Fearon 1992). CD19 is expressed early in B-cell development and required for optimal transition from pro-B-cell to pre-B-cell via successful BCR signalling. The gene *CD19* (encoding the protein CD19) is under epigenetic control by chromatin remodelling allowing the binding of the transcription factors E2A, EBF, and Pax5 to the *CD19* enhancer region (Walter et al. 2008). Epigenetic failure of chromatin remodelling to euchromatin in early B-cell development would result in the failure of the transcription factors E2A, EBF, or Pax5 to bind to *CD19* enhancer and significantly reduce CD19 translation, despite a normal *CD19* DNA sequence. This chromatin remodelling failure would result in a similar clinical CVID phenotype. Over expression of CD19 in acute myeloid leukaemia with *t*(8;21) is due to this translocation increasing PAX5 translation, which acts as a transcription factor increasing CD19 translation (Walter et al. 2010). Therefore a hypothetical scenario in which the opposite occurs and epigenetic downregulation of *CD19* expression may lead to CVID-like phenotype in the context of a normal DNA germ-line sequence.

Epigenetics of Peripheral B-Cell Development and Survival

After successful rearrangement of a functional BCR, immature B cells leave the bone marrow and enter the periphery becoming transitional B cells *en route* to the spleen. Whilst in the spleen, the survival signal B-cell activator of the TNF family (BAFF) binds to the B-cell surface receptors; B-cell maturation antigen (BCMA), transmembrane activator and CAML interactor (TACI), and BAFF receptor (BAFF-R) (Pieper et al. 2013). In this resting stage, B cells express low levels of histone acetylation and large proportions of CpG methylation resulting in inaccessible immunoglobulin heavy chains and no antibody production.

B cells encounter their cognate antigen within lymph nodes and form germinal centres with the help of follicular T helper cells and follicular dendritic cells. Within the germinal centre reaction, B cells undergo class switch recombination (CSR) and somatic hypermutation (SHM). Both of these processes are dependent on the actions of the enzyme activation induced cytidine deaminase (AID) encoded by the gene *Aicda*. DNA demethylation of multiple genes is required for precise gene translation during the germinal centre reaction, and this process partly mediated by AID (Dominguez et al. 2015). *Aicda* transcription is under tight epigenetic regulation due to its potential to induce DNA damage in cells. Naïve, memory

B cells, and plasma cells express low/absent AID, with AID only being expressed within germinal centre B cells undergoing CSR and SHM (Zan and Casali 2015). *Aicda* transcription is regulated by multiple epigenetic elements, including the transcription factors HoxC4, NF-kB, and Sp1/Sp3, ncRNAs, and histone modifications. All these epigenetic controls contribute to the strict regulation of *Aicda* translation demonstrating the importance of AID repression in cells, but also presenting multiple potential candidates for dysfunctional epigenetic repression AID within the germinal centre reaction. The complex network of epigenetic regulation in the germinal centre reaction is reviewed elsewhere (Zan and Casali 2015).

During normal B-cell development, transitional B cells may differentiate into follicular B cells or marginal zone B cells. Following antigenic stimulation marginal zone B cells differentiate into short-lived plasma cells. Follicular B cells differentiate to either memory B cells or long-lived plasma cells and are the predominant dysfunctional effector cell populations in many CVID patients (Pieper et al. 2013). It is the step of antigen-dependent differentiation of follicular B cells to either class switched memory B cells or plasma cells that are defective in CVID resulting in the hallmark of hypogammaglobulinaemia. Each step in B-cell differentiation is guided by multiple epigenetic changes regulating gene expression and giving rise to differing functions of cells in this dynamic adaptive process (Bao and Cao 2016; Zan and Casali 2015).

Following the germinal centre reaction, histone modification and miRNAs silence BLIMP-1 and AID promoting memory B-cell differentiation (White et al. 2014). Plasma cell formation after the germinal centre reaction is regulated by histone and miRNA silencing of other key genes, as reviewed elsewhere (Shen et al. 2015). Epigenetic dysfunction at any stage may, therefore, theoretically result in an arrest or defect in B-cell differentiation and responses (Fig. 2).

MicroRNA Disorders: miRNA-142 Mouse Knock-Out with a CVID-Like Phenotype

The investigation of the clinical impact of ncRNAs in PID is at a very early stage; however, mice models are providing clues to their importance. The miRNA, miR-142, is highly expressed in immune cells, and miR-142 mice knock-out models show phenotypic similarities to CVID with immunodeficiency, hypogammaglobulinaemia, and polyclonal lymphoproliferation (Kramer et al. 2015). In miR-142 knock-out mice, there is increased BAFF-R expression supporting the significance of ncRNAs in the regulation of B-cell function, death, and survival in the peripheral lymphoid organs.

Histone and Chromatin Modification Disorder: Defects in INO80

Recently, a loss-of-function of the E(var) chromatin remodelling complex component INO80 has been shown to cause impaired CSR in B cells and a CVID-like clinical phenotype (Kracker et al. 2015). Immunoglobulin switch regions must be accessible for transcription factor binding during the initiation of CSR. Similar to the mechanisms of B-cell disturbance seen in Kabuki syndrome, defective INO80 causes B-cell CSR defects due to defective histone modification resulting in DNA sequences required for CSR being inaccessible to transcription factor binding. Failure of chromatin remodelling, due to loss of INO80, means that these switch regions remain inaccessible to transcription factors and result in the failure of chromatin undergoing conformational change to euchromatin.

MiRNA-155 Defects: a Possible Dysfunctional Epigenetic Mechanism in CVID

MiR-155 is an miRNA that regulates AID translation, among other functions. Failure of CSR in B cells can present as hyper IgM immunodeficiencies (HIGM) in humans, when the ability to switch immunoglobulin class from an IgM to an alternate immunoglobulin class is defective. Several monogenetic causes for HIGM have been identified (Durandy et al. 2013; Qamar and Fuleihan 2014), but variants in DNA of these genes are not found in all patients with HIGM phenotypes. MiR-155 interacts with AID and disruption of miR-155 impairs CSR which may suggest miR-155 mutation as a cause of undiagnosed HIGM (Bao and Cao 2016). Furthermore, bic/miRNA-155-deficient mice display phenotypic similarities to CVID patients with adaptive immunodeficiency, hypogammaglobulinaemia, lung disease, and enteric inflammation, suggesting a potential role in human CVID (Rodriguez et al. 2007).

Current Evidence of Epigenetics in the Pathogenesis of CVID

Despite extensive molecular studies of CVID patients, there has been minimal investigation of the epigenome in CVID (Flinsenberg et al. 2014; Keller and Jyonouchi 2013; Keller et al. 2014; Li et al. 2015b; Maggadottir et al. 2015; Orange et al. 2011; van Schouwenburg et al. 2015). Tallmadge et al. (2015) recently described abnormal epigenetic regulation leading to repression of key B-cell developmental transcription factors in the cases of equine CVID. In their report late-onset equine CVID was

observed to be due to increased hypermethylation of key genes specifically within B cells. This excessive methylation, or failure of demethylation, resulted in reduced PAX5 expression, B-cell developmental blockade with subsequent reduced B cells, and clinical late-onset CVID. Genomic studies of CVID cohorts using DNA sequencing alone will not detect epigenetic methylation of genes due to the fact that the DNA gene sequencing remains unaltered, but gene transcription does not occur due to epigenetic repression. Such cases would require additional DNA methylation/RNA expression assays for the detection of this abnormality.

Rodríguez-Cortez et al. (2015) reported on a case of monozygotic twins discordant for CVID. This study identified CpG hypermethylation, with failure of demethylation and resultant reduced RNA expression in multiple genes involved in B-cell immunity in the twin with CVID versus the healthy twin and control subjects. Included in this observation was impairment of demethylation of the genes, *PIK3CD, RPS6KB2, KCNN4, KCNC4, CORO1B/PTPRCAP, TCF3,* and *BCL2L1,* specifically between naive to memory B-cell populations (Rodríguez-Cortez et al. 2015). It is worth noting that to detect these abnormal epigenetic changes, analysis must be performed on cells sorted B cells into naïve and memory populations. This is because epigenetic modifications, unlike germ-line DNA changes, can cell subtype specific. The results of the twin study suggested that an accrued epigenetic failure occurred during peripheral B-cell development during naïve to memory B-cell evolution. Of the hypermethylated genes identified, some have been reported to cause a CVID-like PID in the cases of germ-line non-synonymous variations supporting the importance of correct transcription of these genes in B-cell immunity (Angulo et al. 2013; Boisson et al. 2013). Hypermethylated of many of these genes was found only within B cells at specific developmental stages.

Epigenetic plasticity and hypermethylation of specific gene promoters regions during B-cell differentiation, particularly within the germinal centre, is critical to successful B-cell effector function (Hodges et al. 2011). Evidence of failure of DNA demethylation and euchromatin modification in CVID patients and CVID-like disease due to demethylation and histone modification dysfunction suggest that CVID may be an epigenetic disease in patients (Lindsley et al. 2016; Rodríguez-Cortez et al. 2015; Weemaes et al. 2013).

Whole-genome sequencing provides data of the non-protein coding DNA regions and has the ability to detect deleterious variants in ncRNAs that would affect epigenetic regulation which may provide new molecular diagnoses in CVID, but there is a little experience of the impact of variants in non-coding DNA regions (Knight 2013). Limited evidence of the function for some of these vast stretches of non-protein coding DNA only exists in mouse models (Mok et al. 2013; Rodriguez et al. 2007). Other techniques to interrogate CpG methylation status and chromatin immunoprecipitation sequencing in B-cell subsets are also likely to be required for diagnosis in the sporadic cases of CVID.

Epigenetic Treatment Potentials for CVID

Identification of the molecular cause of PID is important due to the increasing availability of precision medical interventions with many of these precision treatments have been repurposed from oncology (Lenardo et al. 2016; Zhang et al. 2015b). Similarly, epigenetic therapies developed within oncology may also be utilised if epigenetic aetiologies for CVID can be elucidated (White et al. 2014; Zahnow et al. 2016).

There are five classes of histone deacetylases (HDACs) based on enzyme structure (Arrowsmith et al. 2012). HDACs are enzymes that remove acetyl groups from histones promoting heterochromatin formation and reduced gene expression. They present therapeutic intervention targets, with many HDAC inhibitors in development and now licensed cancer treatment (Morera et al. 2016). During CSR, access to the switch regions for γ, ε, and α immunoglobulin constant region requires histone alternations, including the demethylation of the histone residues H3K9me3 and H3K27me3, and acetylation of H3K9ac and methylation of H3K4me3 (Kuang et al. 2009; Daniel and Nussenzweig 2012). A failure to perform these histone and chromatin alternations may result in impaired CSR ability within the germinal centre reaction. In mouse models of Kabuki syndrome, HDAC inhibitor treatment improved levels of H3K9me3 suggesting improvement in chromatin remodelling and increased DNA accessibility (Bjornsson et al. 2014). The CVID-like phenotype observed in Kabuki syndrome may, therefore, respond to HDAC inhibition with increased immunoglobubin switch region accessibility during CSR (Vaidyanathan and Chaudhuri 2015).

The DNA hypermethylation in memory B-cell subsets observed in animal and human CVID cases also presents a therapeutic target (Rodríguez-Cortez et al. 2015; Tallmadge et al. 2015). In myelodysplastic syndrome (MDS), DNA hypermethylation has been identified within haematopoietic cell lineages across the genome. Azacitidine is a DNA hypomethylating agent that has shown efficacy in MDS by reducing DNA hypermethylation (Abou Zahr et al. 2015; del Rey et al. 2013; Gurion et al. 2010). The hypermethylation of key B cells genes and promoters described in CVID patients may also be amenable to reduction in DNA methylation and restoration B-cell function and immunity.

Autoimmunity is one of the major causes of morbidity and mortality in CVID patients (van de Ven and Warnatz 2015). The prominent contribution of epigenetic dysregulation now reported in autoimmune conditions has led trials of HDAC inhibitors (Aslani et al. 2016; Szyf 2010). Yet, the observation of varying DNA hypo/hypermethylation and histone status seen in autoimmune conditions warns that a personalised approach to epigenetic treatment is likely to be required in patients with autoimmune conditions and CVID.

As experience of the epigenetics in CVID increases, these therapies may be translated into treatment targets for patients (Egger et al. 2004). Detailed elucidation of epigenetic abnormalities is currently difficult and costly; nevertheless the rapid development of epigenetic treatments and the possibilities these may hold for new treatment avenues in this patient cohort make this worth exploring.

Conclusion

I present an overview of some of the epigenetic regulation of B-cell development and clues to where epigenetic dysfunction may manifest as CVID from animal and human studies. Each stage of lymphocyte differentiation is governed by many more epigenetic interactions and factors then possible to list in this review and many yet to be discovered. Due to the unique requirement of lymphocytes to change their gene expression, morphology, and function throughout cell life, they are susceptible to accruing epigenetic dysfunction with clinical manifestations (Kondilis-Mangum and Wade 2013; Obata et al. 2015).

Progressively accrued detrimental epigenetic changes within lymphocytes, as observed in cancer development, can explain the patient's clinical "progression to CVID". This altered epigenomic hypothesis as an aetiology for CVID is supported lack of a monogenic diagnosis in the majority of cases, often sequential stepwise onset of CVID, and the fact that several monogenetic conditions and CVID-like phenotypes arise due to mutations in genes-encoding components of epigenetic regulation (Kracker et al. 2015; Lin et al. 2015; Lindsley et al. 2016; Weemaes et al. 2013). Germ-line genetic polymorphisms may add relative risk to CVID, but alone these cannot currently entirely explain clinical phenotype due to missing heritability. Further study of the epigenome in CVID patients lacking a monogenic diagnosis may uncover causal mechanisms for disease and identify novel treatments.

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