CHAPTER 8

DOES GENOMIC IMPRINTING PLAY A ROLE IN AUTOIMMUNITY?

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Abstract: In the 19th century Gregor Mendel defined the laws of genetic inheritance by crossing different types of peas.[1] From these results arose his principle of equivalence: the gene will have the same behaviour whether it is inherited from the mother or the father. Today, several key exceptions to this principle are known, for example sex-linked traits and genes in the mitochondrial genome, whose inheritance patterns are referred to as 'non mendelian'. A third, important exception in mammals is that of genomic imprinting, where transcripts are expressed in a monoallelic fashion from only the maternal or the paternal chromosome. In this chapter, we discuss how parent-of-origin effects and genomic imprinting may play a role in autoimmunity and speculate how imprinted miRNAs may influence the expression of many target autoimmune associated genes.

INTRODUCTION

Discovery of Genomic Imprinting

The first evidence for imprinting came more than 25 years ago, from nuclear transfer experiments.[2,3] Mouse embryos were manipulated to contain either two maternal or paternal pronuclei, creating gynogenetic, or androgenetic embryos, respectively. Both sets of embryos failed to develop to term, with the gynogenetic embryos (containing only maternal chromosomes) developing a small embryo but with complete atrophy of extra-embryonic tissues. The androgenetic embryos (containing only paternal chromosomes) were characterised by overgrowth of the extra-embryonic tissues and

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almost total absence of the embryo proper.[2] These pioneering experiments highlighted for the first time that the maternal and paternal genomes in a diploid cell are not functionally equivalent and therefore contain regions whose function is dependent on parental origin. The chromosomal regions of non-equivalence were identified in mice due to the existence of rare cases of uniparental disomy (UPD). UPD is the inheritance of both autosomal chromosomes from one parent and it was observed that inheritance of opposite parental UPDs resulted in different phenotypes that were often reciprocal.[4] It was almost a decade after the nuclear transfer experiments that the first imprinted gene was identified.[5] Since the discovery of *Igf2*, almost 100 imprinted genes have been identified in mice, with around half showing conserved monoallelic expression in humans (www.geneimprint.com). Imprinted genes have been shown to be important regulators of fetal and extra-embryonic growth and neurological development, through controlling cell signaling, cell cycle, metabolism and apoptosis.

Uniparental disomy also occurs in humans and for some human chromosomes are associated with disease due to the presence of imprinted genes on those chromosomes. The clearest example of reciprocal UPDs causing different phenotypes in humans is that of the behaviour syndromes Prader-Willi (PWS) and Angelman syndrome (AS), caused by a maternal or paternal UPD of chromosome 15, respectively.[6,7] It is also well known that UPDs affecting chromosome 14 cause different abnormal growth phenotypes that change according to the parental origin of the UPD.[8] Other examples are Beckwith-Wiedemann syndrome (BWS), where babies are macrosomic and Silver-Russell syndrome (SRS), where the babies are growth restricted. BWS is caused by a paternal UPD of chromosome 11 and SRS by a maternal UPD of chromosome 7.[9,10]

GENOMIC IMPRINTING

Genomic imprinting is the allele-specific expression of a gene depending on its parental origin. To date, most imprinted genes have been identified in mammalian species, however, the phenomenon is also observed in some flowering plants.[11] Since the two copies of autosomes in mammals are identical at the DNA sequence level, the difference in expression must be controlled by an epigenetic mechanism.[12] The term epigenetic refers to heritable changes that do not involve a change in the DNA nucleotide sequence. These include DNA methylation and posttranslational histone modifications that modify the status of chromatin, the molecule that eukaryotic DNA is packaged into. Chromatin consists of nucleosomes, formed by wrapping 146 base pairs of DNA around an octamer of four core histone proteins (H2A, H2B, H3 and H4). Depending on the methylation status of the DNA, combined with certain histone modifications, the chromatin can adopt an active or repressive status, named euchromatin and heterochromatin, respectively.

Genes subject to genomic imprinting constitute a particularly interesting example of epigenetic regulation, since there are active and repressed alleles of the same gene within a single cell. The allelic differences in transcriptional activity originate from the distinct patterns of chromatin structure, due to differential DNA methylation at CpG dinucleotides and covalent histone modifications.[13,14] The allele-specific epigenetic profile of imprinted genes is established in the male and female gametes and maintained throughout somatic development. Regions that display differential DNA methylation (DMRs) in the germ line are referred to as primary imprinting marks. If a DMR has been shown to be indispensable for monoallelic expression in gene targeting experiments, it

is referred to as an Imprinting Control Region (ICR). Commonly, imprinted genes are grouped together in clusters, controlled in cis by a single ICR.

Epigenetic Mechanisms and Imprinting

DNA methylation is an essential modification to DNA, with established roles in gene regulation, genome defense through transcriptional silencing of retrotransposons and genome stability.[15] It is characterized by the transfer of methyl groups to the carbon 5 of cytosine molecules (5-mC) and leads to the recruitment of methyl-CpG binding domain and other transcriptional regulators. Methylated DNA tends to have a closed chromatin conformation and is associated with transcriptionally repressive histone modifications. This contrasts with unmethylated DNA, which is associated with permissive histone modifications and an open chromatin conformation.

DNA methylation is catalyzed by the DNA methyltransferases (DNMTs) that are classified into two families: DNMT1 and DNMT3.[16] The DNMT1 family includes the most abundant DNA methyltransferase in somatic cells, DNMT1, which is responsible for copying DNA methylation patterns to the daughter strands during DNA replication and repair. An oocyte specific DNMT1 (DNMT1o) is involved in maintenance of DNA-methylation at DMRs during early stages of embryo development.[17] The DNMT3 family includes two active forms, DNMT3A and DNMT3B and one regulatory factor, DNMT3-Like protein (DNMT3L). Both DNMT3A and DNMT3B have de novo methyltransferase activity enhanced by DNMT3L[18,19] although DNMT3A is the methyltranferase specifically required for DNA methylation of DMRs in the gametes.[20,21] Recently, it has been suggested that DNMT3 could also be associated with DNA methylation maintenance during DNA replication together with DNMT1.[22]

Histone Covalent Modification

Histone proteins, particularly in their N-terminal tails, are subject to a large number of posttranslational modifications.[23] Acetylation of lysines is generally associated with transcriptional activation. In contrast, the functional consequences of histone methylation, which can occur at lysines (K) and arginines (R), are more dependent on the specific site that is modified. For instance, methylation of H3K4 is closely linked to transcriptional competence, whereas methylation of H3K9 and K20 is associated with transcriptional repression. Further complexity comes from the fact that methylation at lysines can be in the form of either mono-, di- or trimethylation at lysines and mono- or dimethylation (asymmetric or symmetric) at arginines. Histone methylation marks at lysine and arginine residues are relatively stable and can carry epigenetic information from one somatic cell generation to the next.

Regions of differential DNA methylation with imprinted loci are often, but not exclusively, associated with differential chromatin modifications. Methylated alleles are coupled with repressive chromatin modifications such as H3K9me2/3, H4K20me3 and H2AK119u1.[24-27] This heritable repression is due to the coupling of the Polycomb group 1 (PcG) proteins and DNA methyltransferases to form a silencing complex.[28] Unmethylated regions are coupled with permissive chromatin modifications including H3K9ac and H3K4me2/3.[14] Recently it has been shown that certain imprinted genes, not associated with differential DNA methylation at their own promoters, have allelic histone modifications which are required for maintaining somatic imprinting.[24,25,29,30]

These general patterns of histone modifications at DMRs are maintained by the opposing actions of two sets of proteins, the histone acetyltransferases (HAT)/histone deacetylases (HDACs) and the histone methyltransferases (HMT)/histone demethylases (KDM).[31-33] These proteins specifically modify certain histone residues. In fact, the acquisition of differential DNA methylation in the maternal germ-line has recently been shown to require the finely tuned action of the H3K4 histone demethylase AOF1/KDM1B to remove H3K4me before the DNA can become methylated.[34] This process also demonstrates that the biochemical components associated with genomic imprinting are identical to those involved in cell differentiation. This suggests that imprinted regulation does not require unique modifiers to maintain allelic differences in chromatin structure and therefore may be equally prone to epigenetic deregulation during the development of disease states and cancer.

EXAMPLE OF AN IMPRINTED REGION: *H19*/*IGF2* LOCI—AN ANCIENT IMPRINTED DOMAIN

The first imprinted gene to be described was paternally expressed *Igf2*, which is crucial during murine embryogenesis and is implicated in the growth disorders Beckwith-Wiedemann syndrome (BWS) [MIM 130650], Silver-Russell syndrome (SRS) [MIM 180860] and tumorigenesis in humans.[35-37] The *H19/IGF2* locus is the best molecularly characterized imprinted domain in both humans and mice (see Fig. 1). In the mouse, dozens of targeted deletions have delineated the numerous *cis*-acting control elements and in humans characterization has been through the identification of specific epigenetic and cytogenetic defects.[38,39] To date, this gene cluster represents the most evolutionarily ancient imprinted locus identified.[40] The domain has two reciprocally expressed, imprinted transcripts, the maternally expressed, noncoding *H19* gene and the potent growth factor *IGF2*, which is expressed solely from the paternal allele.[41] Although the function of IGF2 as member of the insulin family of peptide growth factors is well known, the function of the *H19* noncoding RNA is still poorly understood.[42] Recently, this alternatively spliced, capped and polyadenylated RNA has been reported to be a pri-RNA for the microRNA miR-675.[40,43] This finding has important implications, as discussed later, suggesting that not all cellular responses due to epigenetic deregulation of this locus are caused by IGF2.

IMPRINTING REGULATION AT *H19*/*IGF2* DOMAIN

The expression of the *H19* and *Igf2* genes is controlled by the differential DNA methylation status of the *H19*-ICR (also known as the *H19* differentially methylated domain or DMD), which is located upstream of the *H19* transcription start site.[44] This ICR is one of the few known paternally DNA methylated ICRs in the genome. Regions of paternal DNA methylation, established somatically after fertilization, are also present at four additional specific sites; one overlapping the *H19* promoter and the remainder, DMR0, DMR1 and DMR2, spread throughout the *IGF2* gene (see Fig. 1).[45] Continued research into the imprinting control of this domain reveals a complicated regulatory mechanism that utilizes multiple enhancers, differentially methylated regions (DMRs), boundary elements, histone modifications and complex

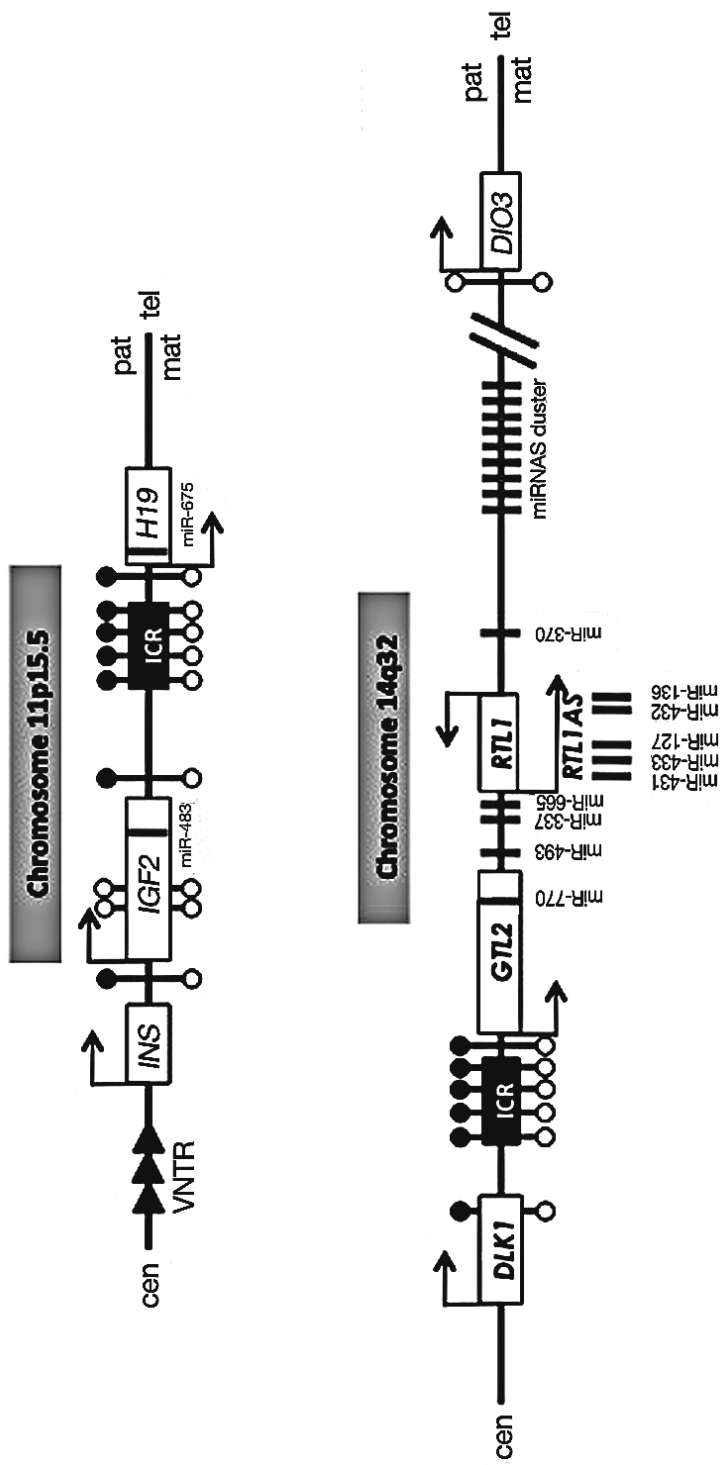

Figure 1. Genomic organization of the *H19-IGF2* and *DLK1-DIO3* imprinted domains on human chromosomes 11 and 14 respectively. The allelic expression in both domains is regulated by regions of paternal-allele DNA methylation. Both regions contain imprinted translated, untranslated and miRNA transcripts.

physical DNA looping, all of which are allele-specific and some species specific.[46,47] Briefly, the paternally DNA methylated germline ICR, located 2-4 kb upstream of the *H19* transcript, contains several CCCTC-binding factor (CTCF) binding sites.[48] These confer the ICR's function as a methylation-sensitive insulator between the multiple *Igf2* promoters and enhancers located downstream of *H19*. On the unmethylated maternal allele, CTCF binds to form a boundary that prevents the *Igf2* promoters interacting with the enhancers, whereas on the DNA methylated paternal allele, CTCF cannot bind and the *Igf2* promoters freely associate with the enhancer to bring about expression from the paternal allele only.[48,49] Using the chromatin confirmation capture technique (3C), it has been shown that on the maternal allele, CTCF binding mediates the formation of a tight, transcriptionally inactive loop around the *Igf2* gene. This involves interactions between the maternal allele of the *H19*-ICR, the matrix attachment region 3 (MAR3)[50] and *Igf2* DMR1, a region previously shown to be a methylation-sensitive silencer. On the paternal allele, the enhancer can form a methylation-sensitive, active chromatin domain through the interaction of the DNA methylated *H19*-ICR allele with the DNA methylated *Igf2* DMR2.[46,47]

Apart from the intrachromosomal interactions at the imprinted *H19-IGF2* locus, mediated by CTCF activity, it is notable that interchromosomal interactions have been reported that involve CTCF binding at the *H19*-ICR. The *H19*-ICR was shown to interact and colocalize with the non-imprinted *Wsb1/Nf1* genes on mouse chromosome 11.[51] This relationship is allele-specific, with the maternal unmethylated allele of the *H19*-ICR associating with the paternal *Wsb1/Nf1* domain. In addition, the maternal allele of the *H19*-ICR was shown to interact directly with the DMRs for the imprinted genes *Impact*, *Kcnq1* and *Napil5*.[52] More recently it has been reported that the *H19*-ICR forms interchromosomal interactions that control expression of several other imprinted genes to form an imprinted gene network, all of which contain CTCF sites.[53,54]

These higher order chromatin loops have been shown to require the sister chromatid cohesion protein, Cohesin. Cohesin binds to the same sites as CTCF, implicating a CTCF-Cohesin complex in regulating gene expression. Utilizing RNAi depletion, Nativio et al, have shown that a lack of SCC1, a cohesin subunit, results in deregulated *H19/IGF2* imprinting, implying that both CTCF and cohesin are required for appropriate monoallelic expression.[55] The CTCF-Cohesin complex is also known to be involved in V(D)J recombination during B lymphocyte development[56] and for appropriate T-helper cell expression of the *IFNG* gene,[57] but it is currently unknown whether the *H19*-ICR interacts with these additional CTCF-cohesin hubs during B- and T-cell differentiation.

AUTOIMMUNITY AND IMPRINTING

Autoimmune diseases are characterised by the failure of self-tolerance and a subsequent immune response against the body's own cells. There are currently eight distinct human phenotypes caused by mutations or epimutations in imprinted genes, with none of these specific disorders sharing features of autoimmune disease. However, as we discuss below, there is evidence that genomic imprinting may play a role in the development and progression of autoimmune disorders.

THE INVOLVEMENT OF IMPRINTED GENES IN TYPE 1 DIABETES

For many years it has been known that both B and T cells contribute to the pathogenesis of autoimmune diseases. It is therefore plausible that any gene that influences B- and T-cell differentiation or function may play a role. The paternally expressed *DLK1* gene maps to the IG-DMR regulated domain on human chromosome 14 (see Fig. 1). *DLK1* is involved in NOTCH dependent signaling that helps transitional B cells develop through cell-cell interactions with stromal cells. Mice that lack *Dlk1* expression have an increased number of early lineage B cells, but a decreased number of recirculated B cells in the bone marrow. In addition, *Dlk1* null mice show abnormal levels of preimmune serum immunoglobulin and an exaggerated antigen-specific humoral immune response.[58] In fitting with a potential role of *DLK1* in autoimmune disorders, DNA association studies using the human Genome-Wide Association (GWA) dataset has shown that paternal inheritance of a rs941576 SNP variant, located within the within the *DLK1-DIO3* locus, is a risk allele for Type 1 diabetes.[59] Insulin Type 1 diabetes mellitus (IDDM) is a multi-system metabolic disease resulting from impaired insulin function, which results in characteristic hyperglycemia and ketoacidosis. Several mechanisms are involved in its pathogenesis, including the delayed-type hypersensitivity reactions mediated by $CD4^+$ TH1 cells that react with islet cell antigens, cytolytic T-lymphocyte mediated lysis of islet cells, production of cytokines TNK and IL-1 that damage the pancreas and production of autoantibodies against islet cells and insulin.

Multiple genes are involved in IDDM, with the majority of attention focusing on the human leukocyte antigen (HLA) genes. HLA genes encode antigen-presenting molecules that initiate T-lymphocyte proliferation after having bound "foreign" peptides and are key in selective loss of B cells. The HLA-DR2 and -DR4 loci are associated with increased susceptibility to IDDM in white Europeans.[60] It has been suggested that the genetics of HLA susceptibility show parent-of-origin effects, with the nontransmitting maternal HLA-DQ2 or -DQ8 alleles being a risk factor,[61] but these observations are disputed.[62,63] Non HLA genes also contribute to the disease. The first to be identified was the insulin gene (*INS)* itself, with the variable number tandem repeats (VNTR) in the 5′-upstream promoter region being associated with disease susceptibility. The *INS* gene is a paternally expressed imprinted gene,[64] and lies next to the paternally expressed *IGF2* gene (see Fig. 1). In rare cases it has been shown that *INS* transcription produces a polycystonic read through transcript that includes the *IGF2* exons,[65] but the function of this transcript is unknown. The expression level of *INS* is regulated by the VNTR. The shorter class I alleles correlate with higher expression in pancreas, but lower levels in thymus.[66,67] These shorter alleles are positively associated with IDDM, while the longer class III alleles are protective.[68] Two studies have suggested that the sensitization to insulin may occur during early life, as a result of ineffective tolerance induction by the decreased expression of insulin in the thymic epithelium in individuals with the VNTR class 1 allele. However, as tantalizing as this theory is, a study in 90 IDDM patients failed to show any association for insulin autoantibody levels with *INS*-VNTR genotype.[69]

PARENT-OF-ORIGIN ASSOCIATION WITH AUTOIMMUNE DISEASES

From the earliest genetic studies on twins, there has been strong evidence for a genetic component in the aetiology of autoimmunity.[70] Much has been learned about the genes involved in autoimmune disease by linkage analyses in families and genome-wide scans. Most autoimmne diseases are polygenic, with individuals inheriting polymorphisms that contribute to disease susceptibility and influence self-tolerance. Most susceptibility loci identified map to large chromosomal domains containing many genes, many of which overlap with regions identified for other numerous autoimmune diseases. Indeed, some HLA alleles within the MHC II region on human chromosome 6 show higher frequencies in various autoimmune patients than in controls. Psoriatic arthritis (PsA) is the combination of two recognised autoimmune diseases, severe arthritis and psoriasis,[71] which shows a less pronounced association with the MHC. Linkage analyses in 906 Icelandic PsA patients show some evidence for imprinted transmission at chromosome 16q. Higher LOD scores were observed when the study was restricted to pairs of affected relatives in whom the last transmission came from the father.[72] This is not the only report of nonMHC linkage in autoimmune phenotypes where LOD scores increased or decreased when the analysis was conditioned on parental transmission; this phenomenon has also been observed for both IDDM and Crohns disease.[73,74] Indeed, analysis of the UK genome-wide scan data revealed evidence for paternal association at D16S3098 in IDDM, which overlaps the region identified for PsA.[75]

IS LOSS-OF-IMPRINTING INVOLVED IN RHEUMATOID ARTHRITIS?

A joint linkage and imprinting analysis performed by Zhou et al on Genetic Analysis Workshop 15 (GAW15) data highlighted rheumatoid arthritis (RA) regions that might be imprinted, but the identified regions failed to withstand additional methods of data analysis.[76] This suggests that genomic imprinting is not involved in RA, however, reports have indicated that loss-of-imprinting (LOI) of *IGF2* occurs in synovial fibroblasts in RA patients[77] but whether this is the cause or consequence of the inflammation is unknown. In rheumatoid arthritis, the synovial membrane, which surrounds the joint space, becomes intensely cellular as a result of immunologic infiltration and increased number of synovial cells. This immune infiltrate contains a large number of T cells, mostly $CD4^+$, that along with other cells express HLA-DR, indicative of activation by inflammation cytokines. These cytokines, that include IL-1 and TNF, are intense stimuli for resident synovial fibroblast (SF) activation. This activation results in the increased proliferation of SF cells to produce a pannus and the production of pro-inflammatory factors and matrix-degrading enzymes that destroy the underlying cartilage and bone.

Using large-scale gene expression profiling, Kasperkovitz et al, showed that RA SF cells show different gene expression profiles depending on the inflammatory status of the tissue from which they are derived.[78] Interestingly, RA SF cells derived from low-inflammatory tissue show high expression of *IGF2*. Subsequent work showed that this increase was due to LOI.[77,78] Thus, the disruption to *IGF2* might be involved in the aetiopathogenesis of RA by increasing the overall expression level of this potent mitogen.

Expression of *IGF2* has also been shown to be involved in aberrant T-cell activation.[79] The expression of *IGF2* in normal mononuclear cells in peripheral blood is imprinted, suggesting that the monoallelic paternal expression is maintained in differentiated

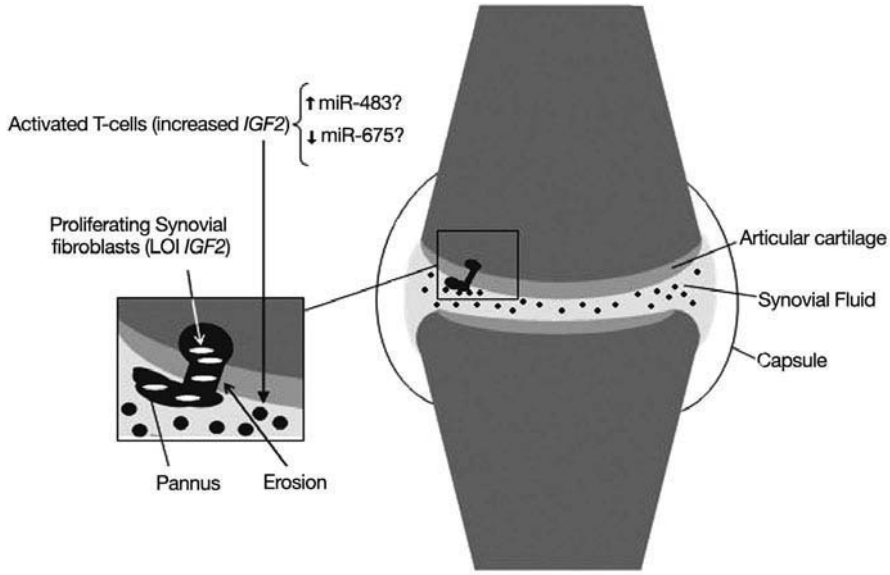

Figure 2. The involvement of *IGF2* in the aetiology of rheumatoid arthritis. Increased *IGF2* expression is observed T-cell activation, but it is currently unknown whether a concurrent increase in miR-483 also occurs. Loss-of-imprinting of *IGF2* is associated with proliferating synovial fibroblats. These cells are responsible for pannus formation and ultimately joint erosion.

hematopoietic cells. However, one study observed LOI in all informative normal bone marrow samples, whereas corresponding peripheral blood shows normal monoallelic expression, suggesting that the high proliferation rates in the bone marrow cells requires a peak of IGF2 to stimulate division.[79] In unstimulated T cells, *IGF2* is monoallelically expressed, however, cultured lymphocytes exposed to PHA show LOI that persists for 72 hours, which results in a two-to six-fold increase of *IGF2* compared to resting T cells. This indicates that careful regulation of *IGF2* expression is required during both expansion in bone marrow, but also in T-cell stimulated proliferation (Fig. 2). However, this LOI of *IGF2* was not observed in lymphocytes isolated from RA patients.[80] It is therefore unclear whether LOI of *IGF2* in RA is maintained after T-cell activation in vivo, however, this mechanism maybe relevant in other, yet to be studied, autoimmune disorders.

FOOD FOR THOUGHT—IMPRINTED miRNAs INFLUENCING AUTOIMMUNE GENES?

MicroRNAs are small noncoding RNA molecules (22-23 nucleotides) that posttranscriptionally regulate gene expression by targeting the 3′ untranslated regions of specific messenger RNAs (mRNA) for degradation or translational repression. miRNA-mediated gene regulation is critical for normal cellular functions such as cell cycle, differentiation and apoptosis and if the process is compromised through genetic ablation of the miRNA machinery or the deregulation of individual miRNA, then this could lead to impaired immunological function and autoimmunity.

Table 1. A comprehensive list of imprinted miRNAs that potentially regulate genes involved in autoimmune diseases. All the miRNA-target gene interactions are catalogued in the TargetScan and miRBase databases

Autoimmune Disease	Candidate Gene	miRNA	miRNA Region
CeD	HLA-DQA1	miR-665	*DLK1-DIO3* (14q32)
MS	HLADRB1		
	HLA-B	miR-483	*IGF2-H19* (11p15.5)
Psoriasis	HLA-C	miR-665	*DLK1-DIO3* (14q32)
		miR-370	
	LCE3D	miR-296	*GNAS* (20q13.3)
Crohn's	IL23R		
	NOD2	miR-483	*IGF2-H19* (11p15.5)
		miR-431	*DLK1-DIO3* (14q32)
	CCR6	miR-433	
	TNFSF15	miR-127	
		miR-433	
		miR-432	
	CDKAL1	miR-335	*MEST* (7q32.2)
		miR-432	*DLK1-DIO3* (14q32)
		miR-296	*GNAS* (20q13.3)
RA	PTPN22	miR-335	*MEST* (7q32.2)
	HLADRB1	miR-665	*DLK1-DIO3* (14q32)
SLE	HLA-DQA1		
	BLK	miR-298	*GNAS* (20q13.3)
T1D	PTPN22	miR-335	*MEST* (7q32.2)
	C10orf59	miR-665	*DLK1-DIO3* (14q32)
	CTLA4	miR-432	
		miR-493	
	IL27	miR-296	GNAS (20q13.3)

It is estimated that almost 30% of all mRNAs are regulated by miRNAs, with each miRNA having multiple target mRNAs.[81] Additionally, roles for miRNAs in antigen receptor expression and successful lymphocyte-restricted gene expression are emerging.[82] A recent analysis of predicted miRNA-mediated regulation of 72 Lupus susceptibility genes in humans revealed numerous target sites for over 140 miRNAs conserved in mammals. These findings highlight the physiological need to control final protein products with enormous precision to maintain the balance between immunity and tolerance.[83] Overlap amongst targets of individual miRNAs is considerable, with the 11 miRNAs within the *DLK1-DIO3* imprinting cluster predicted to regulate 48 systemic lupus erythematosus (SLE) susceptibility genes. Indeed, this observation is not just limited to SLE, as when Royo et al extend the analysis to include all 19 miRNAs that map to imprinted loci,[84] it becomes evident that these imprinted miRNAs have seed target sites in many autoimmune associated genes, relating to many disorders (Table1).

CONCLUSION

A role for imprinted genes in B- and T-cell development and activation is becoming evident, however more research is required to confirm a direct role in the aetiology of autoimmune diseases. Recent studies have identified numerous imprinted miRNAs, the tissue-specific mRNA targets of which still have to be deciphered. It is therefore possible that any epigenetic disruption to the imprinting mechanism will affect the allelic expression of not only imprinted mRNAs but also the miRNAs, with the knock-on effect of altering the fine balance of their target gene expression in trans.

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