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Epigenetics and complementary proteins

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Summary

Although studies on the immunopathogenesis of anti-neutrophil cytoplasm antibody (ANCA) vasculitis have been directed at understanding the autoantibody, there is growing evidence that points to the importance of ANCA autoantigen genes and their regulation. Transcriptional analysis indicates that ANCA autoantigen genes are active in mature neutrophils of ANCA vasculitis patients compared to healthy controls. The unusual transcriptional state of neutrophils from ANCA vasculitis patients appears to be a consequence of failed or disrupted epigenetic silencing. Defective epigenetic silencing could have global effects, by altering the transcriptional and phenotypic state of neutrophils, or local effects by permitting transcription of autoantigen genes from both strands resulting in anti-sense transcripts. Although the role of anti-sense transcripts is currently unknown, there are two intriguing possibilities. Anti-sense transcripts could function (as described for other genes) in transcriptional silencing of autoantigen genes, which takes place in normal neutrophil progenitors. In the setting of failed epigenetic silencing, the fate of anti-sense transcripts may be pathological and serve as a template for production of complementary autoantigens. The observation that ANCA vasculitis patients have anti-sense transcripts and antibodies to complementary proteins is consistent with a role of anti-sense transcripts in complementary protein production. A better understanding of epigenetic silencing and complementary proteins in ANCA vasculitis may unlock the underlying pathology of this condition.

Keywords: anti-sense transcripts, complementary proteins, epigenetic silencing, histone methylation, vasculitis

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Research on the immunopathogenesis in anti-neutrophil cytoplasm antibody (ANCA) vasculitis has focused primarily on the identity, origin and role of the autoantibody, with little effort directed at understanding the role of the autoantigen. The molecular mechanisms responsible for the immunopathogenesis have not been unravelled completely, but clinical and experimental evidence indicates that ANCA cause vascular injury by activating neutrophils [1–5]. Despite data demonstrating that the presence of ANCA are sufficient to cause disease, a number of factors can aggravate the disease process, including environmental pressures, suggesting that ANCA vasculitis escalates in patients who harbour an additional defect(s). A fascinating possibility is that the additional defect(s) contributes to the development of the autoantibodies.

One candidate for an additional defect among ANCA vasculitis patients is dysregulated gene expression, leading to different transcriptional profiles of neutrophils from ANCA patients compared to neutrophils from healthy controls. Importantly, two major ANCA autoantigens, granule proteins proteinase 3 (PR3) and myeloperoxidase (MPO), which are expressed predominantly during the myeloblast and promyelocyte stages of neutrophil development [6], are expressed aberrantly in mature neutrophils of ANCA patients contrasted with their normally silenced state in mature neutrophils of healthy controls [7,8]. The aberrant expression of *PR3* and *MPO* genes in mature neutrophils from ANCA patients is probably a consequence of defective epigenetic silencing [9].

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Support for an epigenetic silencing defect among ANCA vasculitis patients comes from chromatin immunoprecipitation (ChIP) experiments which showed that the *PR3* and *MPO* genes in neutrophils of ANCA patients relative to healthy controls are depleted of H3K27me3, a mark of transcriptionally silent chromatin. The establishment and maintenance of this epigenetic silencing mark results from the activities of a specific histone modifying complex (PRC2) and its methyltransferase subunit (EZH2), and histone demethylases (JMJD3 and UTX) specific for H3K27me3. In ANCA vasculitis patients the balance of histone methyltransferase and demethylase activity tilts away from silencing because there is a failure to recruit PRC2 and EZH2 to the *PR3* and *MPO* genes, and there is increased expression of JMJD3 and UTX. A further epigenetic silencing defect involving DNA methylation has also been identified in ANCA patients. A CpG island in the *MPO* gene remains unmethylated in ANCA vasculitis patients while methylated in healthy controls. These data suggest that epigenetic dysregulation is an important defect in ANCA vasculitis.

There are several potential consequences of epigenetic dysregulation. The aberrant expression of *PR3* and *MPO* may be a surrogate marker for global epigenomic changes in neutrophils of ANCA patients. The global epigenomic changes manifest in distinct transcriptional profiles for neutrophils of ANCA patients [10], which may signify a neutrophil that responds more severely to autoantibodies. A second consequence of the epigenetic defect could be specific to *PR3* and *MPO* genes because the failure to silence these autoantigen genes in mature neutrophils provides a template for inappropriate production of these antigens. Inappropriate expression of *PR3* and *MPO* may alter the availability of these antigens by targeting these proteins to granules that are exocytosed more readily. Finally, the defect in epigenetic gene silencing could provide an accessible or even permissive chromatin template for anti-sense transcription.

Large RNA sequencing and transcriptome projects have identified uncharacterized long non-coding RNAs surpassing the number of known coding transcripts [11,12]. Some of the more well-known long non-coding RNAs, such as *Xist*, *Air* and *Kcnq1ot1*, function to regulate gene expression. A speculative model for transcriptional regulation of *PR3* and *MPO* would posit a role of anti-sense *PR3* or *MPO* transcripts in targeting silencing complexes, such as PRC2, to *PR3* or *MPO* loci, respectively. For instance, during normal neutrophil development the gradual accumulation of anti-sense *PR3* transcripts in promyelocytes would help to target PRC2 to the *PR3* gene. Targeting PRC2 would establish H3K27me3 and transcriptional silencing of the *PR3* locus (both sense and anti-sense DNA strands) observed in mature neutrophils. In ANCA patients, according to this model, anti-sense transcripts that accumulate in promyelocytes fail to establish and/or maintain H3K27me3 because either PRC2 is not recruited properly or the demethylase activity of JMJD3 and UTX erases methylation of H3K27. In mature neutrophils of ANCA patients the *PR3* locus could then be transcribed from both strands.

The presence of anti-sense transcripts in mature neutrophils of ANCA patients could be more significant than an additional marker of disrupted epigenetic silencing. Anti-sense transcripts could be templates for the production of complementary proteins. Long non-coding RNAs identified recently in RNA sequencing projects are thought to be 'non-coding' based on computational analyses; however, these methods 'cannot formally reject the hypothesis that some "non-coding" RNAs are translated *in vivo* or in cell lines under biological conditions' [13]. With this in mind, in the case of ANCA vasculitis the presence of the anti-sense *PR3* transcript would be an endogenous template for the production of complementary PR3 protein. As documented previously, ANCA patients have antibodies that recognize complementary PR3 protein, and it has been hypothesized that autoantibodies against sense PR3 protein are generated through an antibody (cPR3)-idiotypic antibody (sense-PR3) network [14]. Evidence in favour of this hypothesis for autoimmunity comes from studies demonstrating that some patients with ANCA vasculitis have T cells that respond to complementary PR3 protein [15], and the *HLA DRB1(*)1501* allele found in African Americans with PR3-ANCA encodes a major histocompatibility complex (MHC) class II receptor that binds sense and complementary PR3 [16]. To date, there are few additional data supporting this hypothesis; in fact, there are some contradictory data (Peter Heeringa, personal communication).

We have detected an anti-sense *PR3* transcript in ANCA patients (unpublished data), but whether it serves as a template for complementary PR3 protein awaits experimental validation. If, in fact, it is confirmed that complementary PR3 can be produced from the anti-sense *PR3* transcript, questions still remain: is there a role for the anti-sense *PR3* transcript in silencing of sense *PR3*? Would silencing of sense *PR3* occur post-transcriptionally via an RNAi mechanism, or at the transcriptional level, as suggested by the model discussed above? Is the primary defect in epigenetic silencing or is the anti-sense transcript sequestered by the translation machinery and unavailable to initiate silencing? Is there an anti-sense *MPO* transcript, and does it function similarly? By addressing these questions, we may answer the more profound question of whether the regulation of the autoantigen is the key to immunopathogenesis in ANCA vasculitis.

Disclosure

None.

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