Editorial Comments

UGA hopping: a sport for nephrologists too?

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Nonsense mutations and translational read-through

The genetic information encoded by DNA is ultimately expressed into specific polypeptides by way of transcription and translation. Transcription is the process whereby the coding gene sequence is transferred into a messenger RNA (mRNA). Through translation, polypeptides are synthesized at the ribosomes, collinearly with the sequence of codons in mRNA. Codons are sets of three ribonucleotides in mRNA that signal the initiation, elongation and termination stages of translation. With the exception of the nonsense codons (UAG, UAA and UGA), all codons have corresponding complementary anti-codons in transfer RNA (tRNA) molecules. Each tRNA carries a specific amino acid, which is incorporated into the elongating polypeptide according to the codon sequence in mRNA. The correct mRNA reading frame is defined at the start of translation by the start codon (AUG), which codes for methionine. Termination of translation is normally signalled by the occurrence of any of the three codons for which no tRNA is available, hence named stop codons. Protein release factors specifically bind to stop codons, mediating the release of the newly synthesized polypeptide from the ribosome. However, the stop codons are not equally effective in determining the efficiency of translation termination, with the relative rank order of efficiency being UAA > UAG > UGA [1]. When translational reading through stop codons occurs, UAA and UAG usually direct the addition of glutamine into the nascent polypeptide, whereas tryptophan is incorporated in response to UGA in bacteria [2].

Nonsense mutations are single-nucleotide alterations in the DNA that directly change sense to nonsense codons, causing a premature stop of the mRNA translation process. Stop codons may also be generated by mutations that alter the reading frame of mRNA, either as a result of insertion and/or deletion of a number of nucleotides not evenly divisible by three, or of an abnormally spliced RNA. Premature termination codons (PTC) lead to the formation of truncated proteins that do not function properly. The frequency of PTCs is rather variable, ranging from 5% to 70% of reported mutations for different diseases [3–5]. Cellular defence mechanisms against the potential deleterious consequences of PTCs involve the degradation of the truncated proteins, and the detection and degradation of mRNAs containing these codons through nonsense-mediated mRNA decay (NMD) (Figure 1). NMD is a ribosomal function that scans newly synthesized mRNAs for the presence of PTCs in a pioneer round of translation [6,7].

Normal stop codons are surrounded by upstream and downstream sequences, which enhance the efficiency of translation termination [8,9]. As PTCs lack these surrounding complementary signals, pharmacological approaches may be devised that allow translational read-through of in-frame PTCs, while keeping the physiological stop codon active, thus potentially yielding a full-length normal or functional protein (Figure 1) [10].

From nephrotoxic parenteral antibiotics to safe orally available drugs

Two translational read-through therapies for in-frame nonsense mutations have already been tested in human clinical trials: aminoglycosides and ataluren.

Aminoglycosides make ribosomes read through PTCs, thereby generating full-length proteins. In cell culture, aminoglycosides allow reading through human mutations causing X-linked nephrogenic diabetes insipidus, nephropathic cystinosis and polycystic kidney disease [10,11]. Variability in the response was found in clinical trials on cystic fibrosis patients [10,12]. However, current aminoglycosides are not clinically useful to treat human kidney genetic diseases since potentially nephrotoxic concentrations are needed in vivo. In addition, gentamicin upregulated several mRNAs that are known to be NMD

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substrates [13]. This might generate further adverse effects since NMD is thought to be involved in the regulation of gene expression and inactivation of NMD results in altered expression of 10% of all cellular mRNAs [14]. Targeting the NMD carries a risk of increasing the amounts of mRNAs normally degraded by NMD [5]. A better understanding of the molecular mechanisms, underlying aminoglycoside selectivity for prokaryotic ribosomes as well as of the human toxicity, eventually leads to the design of less toxic and more efficient molecules to treat genetic diseases [15].

Ataluren (PTC124) belongs to a new family of oxadiazole compounds with no structural similarity to aminoglycosides [13]. Ataluren was identified in a high-throughput screen for compounds that promoted suppression of UGA stop codons [13]. Ataluren also promotes read-through UAG and UAA stop codons, albeit less efficiently [13]. Similar to aminoglycosides, in the presence of ataluren, the PTCs are ignored, the real stop signals are read and the amount of full-length protein increases. However, truncated protein will still be synthesized, and theoretically, the full-length protein may have either the correct or an abnormal amino acid. Contrary to aminoglycosides, ataluren does not interfere with NMD [13]. Pre-clinical data indicated no changes in transcription or enhanced stability of CFTR mRNA, and the proportion of CFTR mRNA transcripts that contained a nonsense mutation was unchanged in clinical trials [13,16]. Ataluren therapy resulted in production of full-length dystrophin in mdx mice and restored the functional activity of human PTC-bearing CFTR transcripts in a murine cystic fibrosis model [13,17].

Single doses of oral ataluren were well tolerated in phase I safety studies [18]. Phase II safety and efficacy clinical trials have been completed for cystic fibrosis (frequency 1 in 2000) and Duchenne muscular dystrophy (frequency 25 per 100 000 male live births). Recruitment is ongoing for a phase II trial in haemophilia and phase III trials in cystic fibrosis [19]. In a cystic fibrosis phase II trial, ataluren for 2 weeks improved the nasal chloride channel defect in 17 out of 23 (74%) patients, most of them carrying an UGA stop codon [16]. Lung function significantly improved, but the sweat chloride channel defect was not corrected. The clinical response was variable and more evident in patients with a higher cellular concentration of mutant CFTR transcripts. However, there is an insufficient understanding of the variability in the clinical response [16].

**Potential application in kidney disease**

Many hereditary diseases with kidney involvement may be caused by nonsense mutations (Table 1) and may be potential beneficiaries of translational read-through approaches. Diseases and individuals most amenable to therapy will be those in which low levels of physiologically functional proteins are sufficient to restore their function and enough levels of PTC-containing mRNAs are present [10].
Autosomal dominant polycystic kidney disease (ADPKD) is the most common renal hereditary nephropathy, with an estimated frequency of 1 in 800. Although \( \sim 70\% \) of PKD1 and 80\% of PKD2 mutations are predicted to be truncating, only 25\% and 29\%–33\%, respectively, are nonsense in-frame and point mutations [20,21]. The role of read-through strategies in ADPKD remains unknown as does the pathogenesis of the disease regarding its genetic basis. It is not absolutely known whether haploinsufficiency or a dominating negative effect is the underlying problem.

In ARPKD, 11\% of mutations are nonsense [21]. However, this percentage raised to 23\% in a recent series of patients with more severe disease, fetuses and neonates who died shortly after birth [22]. Consideration of read-through strategies in the latter setting is complicated by potential side effects during pregnancy.

About 50\% of mutations in Alport syndrome are nonsense involving the amino acid glycine. However, a significant percentage of disease-causing mutations are PTC caused by nonsense mutations [21,23]. In these cases, read-through strategies could allow the synthesis of sufficient amount of collagen 4 type 3, 4 or 5 to maintain the functional structure of the glomerular basement membrane.

Although Fabry disease can be treated by enzyme replacement therapy (ERT), this must be administered intravenously every 2 weeks, and is very expensive and of a questionable therapeutic efficacy in clearing the accumulated substrate from some kidney cell types [24]. Nonsense mutations affecting CpG dinucleotides are among the most common mutations causing the classic phenotype of Fabry disease [25]. In these patients, translational read-through therapies may provide a means to reduce dependence on ERT. Their ability to clear deposits from key cell types should be explored if cell culture studies provide evidence of efficacy.

Regarding exploratory experiments on the effect of read-through strategies on protein function in vitro is easy in Fabry disease, where enzymatic activity is tested. However, in vitro testing of the functional efficacy of translational read-through may be very difficult in some inherited kidney disorders. In vivo adequate and sensitive biomarkers should be identified to follow up the effect of therapy.

### Potential risks

Ataluren has been safe in short-term trials carried up to now, but potential untoward effects must be monitored in longer trials [16]. There is a theoretical possibility that read-through strategies awake retrotransposons and endogenous retroviruses disabled by PTCs [26]. However, ataluren does not suppress mRNAs containing more than one PTC, as it is typical for retrotransposons [13]. Potential modulation of human polymorphisms or acquired changes resulting from PTCs in the human population

### Table 1. Nonsense mutations in hereditary disease with renal involvement

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Gene symbol</th>
<th>% nonsense mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystic diseases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD polycystic kidney disease</td>
<td>PKD1</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>PKD2</td>
<td>29</td>
</tr>
<tr>
<td>AR polycystic kidney and hepatic disease</td>
<td>PKHD1</td>
<td>11</td>
</tr>
<tr>
<td>Nephronophthisis</td>
<td>INVS</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>NPHP1</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>NPHP3</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>NPHP4</td>
<td>17</td>
</tr>
<tr>
<td>Hyperuricaemic nephropathy, juvenile/medullary cystic kidney disease 2</td>
<td>UMOD</td>
<td>0</td>
</tr>
<tr>
<td>Tuberous sclerosis</td>
<td>TSC1</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>TSC2</td>
<td>16</td>
</tr>
<tr>
<td>von Hippel–Lindau syndrome</td>
<td>VHL</td>
<td>8</td>
</tr>
<tr>
<td>Alport syndrome/type IV collagen nephropathy</td>
<td>COL4A3</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>COL4A4</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>COL4A5</td>
<td>6</td>
</tr>
<tr>
<td>Alport syndrome with macrothrombocytopenia</td>
<td>MYH9</td>
<td>7</td>
</tr>
<tr>
<td>Hereditary nephrotic syndrome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nephotic syndrome/congenital nephrotic syndrome, Finnish type</td>
<td>NPHS1</td>
<td>10</td>
</tr>
<tr>
<td>Nephotic syndrome, steroid resistant</td>
<td>NPHS2</td>
<td>7</td>
</tr>
<tr>
<td>Nephotic syndrome 3, early onset</td>
<td>PLCE1</td>
<td>57</td>
</tr>
<tr>
<td>Nephotic syndrome/Pierson syndrome</td>
<td>LAMB2</td>
<td>36</td>
</tr>
<tr>
<td>Denys–Drash syndrome</td>
<td>WT1</td>
<td>16</td>
</tr>
<tr>
<td>Nephotic syndrome/Schimke immuno-osseeus dysplasia</td>
<td>SMARCA1L</td>
<td>29</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lecithin:cholesterol acyltransferase deficiency/fish-eye disease</td>
<td>LCAT</td>
<td>3</td>
</tr>
<tr>
<td>Fabry disease</td>
<td>GLA</td>
<td>10</td>
</tr>
</tbody>
</table>

Data are expressed as percentage of reported genotypes involving nonsense mutations (Human Gene Mutation Database, http://www.hgmd.cf.ac.uk/ac/index.php). AD, autosomal dominant; AR, autosomal recessive.
may be expected. Thus, the human caspase-12 gene is polymorphic for the presence or absence of an UGA stop codon [27]. Most humans carry this PTC, resulting in a shorter, inactive protein. The predominance of the inactive form is thought to result from its selective advantage conferring sepsis resistance. In this regard, one of the patients involved in the phase II cystic fibrosis trial discontinued the study because of exacerbation of a pre-existing Mycobacterium abscessus infection [16].

**Future directions**

Translational read-through is a promising approach for the treatment of hereditary diseases that may complement the use of chaperones for missense mutations. Ataluren is currently undergoing phase III clinical trials in non-renal diseases. The characterization of its molecular target will allow the development of a new generation of drugs. The application of these compounds in kidney disease requires a pre-clinical evaluation of their potential in mutation-carrying cell cultures. The aim would be to develop read-through ‘tailor made’ therapies based on the sequence profile of each individual. Therapeutic approaches aiming to specifically increase the level of transcripts carrying the disease-causing PTC may increase the response to read-through therapy [10].

The wide ranging implications of translational read-through technology for subgroups of patients with rare diseases in which formal clinical trials are not feasible may generate legal issues, and some common pre-clinical criteria that predict potential clinical response in humans should be devised [28,29].

Mutation-targeted therapies, of which translation reading-through compounds for in-frame nonsense mutations and antisense oligonucleotides for splicing mutations [30] have reached clinical trials, may soon become a new paradigm for the treatment of human genetic diseases by correcting the effect of mutations at the DNA or RNA level, rather than at the protein level. The availability of these therapies should be devised [28,29].

**Supplementary data**

Supplementary data is available online at http://ndt.oxfordjournals.org.

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Can a dysregulated mucosal immune system in IgA nephropathy be controlled by tonsillectomy?

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Keywords: IgA nephropathy; macroscopic haematuria; mucosal immune system; tonsillectomy

Introduction

Even though IgA nephropathy (IgAN) is by definition a diagnosis requiring renal biopsy tissue examination in immunohistology, a well-grounded suspicion is possible in the presence of macroscopic haematuria, detected coincidentally or immediately following an upper respiratory or gastrointestinal tract infection. Many years ago, it was suggested that this hallmark of IgAN supported the hypothesis that the mesangial deposits of polymeric IgA detected by immunohistology are due to a mucosal immune system response to environmental pathogens [1]. Mucosal (or innate) immunity acts through recognition of pathogen-associated molecular patterns by Toll-like receptors (TLRs) expressed on phagocytic cells favouring virus or bacteria removal. The activation of TLRs induces dendritic cell maturation and migration to lymph nodes, leading to activation of specific T-cells and antibody synthesis, thus promoting a link between innate and adaptive immunity played at the mucosal and systemic level [2].

However, the discrepancy between the high incidence of viral syndromes in the general population and low prevalence of IgAN suggests that abnormalities of the mucosal and/or systemic immune system are critical for the development of IgAN, with infections representing a triggering event. Repetitive exposure to various infectious agents has been proved to induce experimental IgAN in animals only after abrogation of the natural process of mucosal tolerance which favours host defence after protracted pathogen exposure [3]. According to the hypothesis concerning the role of detective mucosal tolerance, patients with IgAN should have impaired elimination of mucosal antigens leading to continuous antigenic challenge, which triggers the production of nephritogenic IgA. Polymeric IgA molecules deposited in the mesangium in IgAN are mostly of IgA1 subclass, and present with a defective glycosylation and a reduction of galactose and/or N-acetylgalactosamine residues [4]. Also, in normal subjects, poorly galactosylated IgA1 circulates in the bloodstream after immune response to mucosal antigens or mucosal vaccines. Increased presence of degalactosylated IgA1 in the circulation is presently considered due to the misdirection of mucosal IgA1-committed plasma cells (with increased activity due to unknown mechanisms) to secrete mucosal type IgA1 into the circulation [5]. There, poorly glycosylated IgA1 undergoes formation of macromolecules due to self-aggregation or the reaction with antigens or IgG antibodies directed towards these glycoforms.

To sum up, the dysregulation of innate immunity in IgAN is likely to result in failure of mucosal antigen elimination and/or altered IgA1 synthesis and secretion. There is no need to postulate the action of peculiar antigens, as common microbial or food antigens may play this role. A variety of pathogens have been used in experimental models of IgAN, including Staphylococcus aureus in Th2-prone mice [6], oral immunization with Haemophilus parainfluenzae and repetitive intranasal immunization with Sendai virus [3]. Besides pathogens, gliadin or other com-