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Review

Regulation of the expression of inducible nitric oxide synthase

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ABSTRACT

Nitric oxide (NO) generated by the inducible isoform of nitric oxide synthase (iNOS) is involved in complex immunomodulatory and antitumoral mechanisms and has been described to have multiple beneficial microbicidal, antiviral and antiparasital effects. However, dysfunctional induction of iNOS expression seems to be involved in the pathophysiology of several human diseases. Therefore iNOS has to be regulated very tightly.

Modulation of expression, on both the transcriptional and post-transcriptional level, is the major regulation mechanism for iNOS. Pathways resulting in the induction of iNOS expression vary in different cells or species. Activation of the transcription factors NF-κB and STAT-1α and thereby activation of the iNOS promoter seems to be an essential step for the iNOS induction in most human cells. However, at least in the human system, also post-transcriptional mechanisms involving a complex network of RNA-binding proteins build up by AUF1, HuR, KSRP, PTB and TTP is critically involved in the regulation of iNOS expression. Recent data also implicate regulation of iNOS expression by non-coding RNAs (ncRNAs).

Contents

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Introduction

Nitric oxide (NO), one of the smallest known bioactive products of mammalian cells, can be produced by almost all cells. In mammals, three distinct isoforms of NOS (neuronal (n)NOS, inducible (i)NOS and endothelial (e)NOS) have been identified. These enzymes are products of different genes, with different localization, regulation, catalytic properties and inhibitor sensitivity, and with 51–57% homology between the human isoforms [1]. nNOS and eNOS are primarily expressed in neurons and endothelial cells, respectively. These isoforms are low output Ca^{2+}-dependent enzymes producing NO in a pulsative manner. iNOS is a high output Ca^{2+}-independent NOS whose expression can be induced in a wide range of cells and tissues by cytokines and other agents. After induction, iNOS continuously produces NO until the enzyme is degraded [2].

The high amounts of NO produced by iNOS can have beneficial microbicidal, antiviral, antiparasital and antitumoral effects [3]. In contrast, aberrant iNOS induction may have detrimental consequences and seems to be involved in the pathophysiology of multiple human diseases such as asthma, arthritis, multiple sclerosis, colitis, psoriasis, neurodegenerative diseases, tumor development, transplant rejection or septic shock [4,5].

In view of this multitude of effects and functions of iNOS-produced NO, it is clearly important to understand the mechanisms by which cells accomplish and regulate their iNOS-related NO production. As NO production by iNOS is mainly regulated by modifying the expression of iNOS, this review will concentrate on the description of the mechanisms regulating iNOS expression. In addition, as regulation of iNOS expression in human cells is quite complex this review will mainly concentrate on data obtained using human cell systems.

Structure of the human iNOS gene

The human iNOS gene (NOS2; GeneID 4843; ENSG00000007171; NCBI NC_000017.10) is located on chromosome 17q11.2-q12 (Chromosome 17: 26,083,792–26,127,555 reverse strand; see Fig. 1). The gene is build up by 43,764 bp. According to the definitions of the ENSEMBL database, the human NOS2 gene codes for 2 transcripts: NOS2_001 (ENST00000313735) and NOS2-201 (ENST00000379105). The NOS2_001 transcript (NM_000625.4) contains 27 exons of the human NOS2 gene, is 4176 nt in length and codes for a protein with 1153 amino acids. This transcript is believed to be the major human iNOS mRNA. The NOS2_201 (NM_153292.1) transcript, which is a splice variant of NOS2_001, contains 28 exons of the human NOS2 gene, is 4089 nt in length and codes for a protein with 1114 amino acids. However, until now there is insufficient support for the expression of the NOS2_201 transcript and protein.

Partly overlapping with the 3′-UTR of the human iNOS there is a putative pseudogene (LOC645754) of the galectin-9-like protein B (LGALS9C) on the opposite strand of the iNOS locus (see Fig. 1). If this sequence is expressed and may regulate iNOS expression in human cells by an antisense mechanism (analogous to the rat system, see below) has not been analyzed.

Comparison of the human iNOS gene locus with the gene loci of the mouse, rat, rhesus macaque and chimpanzee genome shows high homology between the primate sequences (as there are some unsequenced regions in the chimpanzee and rhesus macaque genome in the iNOS gene locus, these regions show no homology; see Fig. 1). In contrast the homology between the human, rat and mouse sequences is relative low (highest homology in the exon sequences). However, there are some spots of high homology (evolutionary conserved regions – ECR) both in the 5′- and the 3′-flanking genomic sequences. It is likely that these sequences are involved in the regulation of iNOS expression.

There are several sequences with high homology to the bona fide iNOS genes (NOS2) in the human genome [6,7]. In a recent paper Korneev et al. described regulation of iNOS expression by one on these sequences by an antisense mechanism (see below) [8].

Regulation of iNOS activity

The iNOS is primarily regulated at the expressional level by transcriptional and post-transcriptional mechanisms. In contrast to nNOS and eNOS, regulation of enzyme activity is unusual for iNOS. However Hausel et al. described regulation of iNOS activity (and protein stability, see below) by src-mediated tyrosine phosphorylation [9].

As iNOS activity depends on arginine availability, regulation of arginine transport [10,11] or consumption of arginine by other biochemical pathways (for example arginase [12–15]) has been shown to regulate iNOS enzyme activity.

The active iNOS enzyme is a homodimer. Homodimerization of iNOS depends on the availability of its essential cofactor tetrahydrobiopterin (BH4) [16,17]. Therefore mechanisms regulating BH4 synthesis and consumption regulate iNOS activity [18,19].

Additionally some proteins have been identified that interact with iNOS and regulate its activity. By yeast two hybrid screens using murine iNOS as bait the protein kalirin was shown to interact with the iNOS protein. This protein inhibits iNOS activity by preventing enzyme dimerization [20]. In murine macrophages, a 110-kDa protein (named NAP110) has been identified, that directly interacts with the amino terminus of iNOS, thereby preventing dimer formation and inhibiting NOS activity [21]. In rat vascular smooth muscle cells iNOS protein has been found to interact with the calcium/calmodulin-dependent protein kinase II (CaMKII) [22]. Beside regulation of cellular iNOS protein localization all