The nuclear receptor superfamily – Structure, functions and therapeutic aspects

Nuclear receptors are ligand-inducible transcription factors, which are a family of proteins characterized by two important segments of amino acid sequence which are highly conserved across all subfamilies of these receptors – the DNA binding domain (DBD) and the ligand binding domain (LBD). Hence, their two major functions involve binding small and specific sets of DNA sequences via DBD and binding specific ligands via LBD through which they regulate transcription. Examples of nuclear receptors include estrogen receptor, progesterone receptor, androgen receptor, glucocorticoid receptor, Vitamin A receptor, Vitamin D receptor, and thyroid hormone receptor (Sladek, 2010). This paper aims to provide an overview of the history, superfamily, structure and functions of the nuclear receptors based on the information we have at present.

The initial discovery of nuclear receptors is attributed to their ability to bind specific ligands (Germain et al., 2006). The estrogen receptor was the first to be identified by Jensen and his colleagues. In order to understand how the estrogen hormone exerted its effects in the tissue, they decided to study the biochemical mechanisms of hormone function. This revealed the presence of certain hormone-binding components leading to the identification of the first nuclear receptor (Khan and Lingrel, 2010). After the initial discovery, subsequent receptors were identified based on their sequence similarity with the first identified receptors (Naar and Thakur, 2009). The receptors discovered by this technique, which did not have any specific ligand attributed to them, were known as orphan receptors (Sladek, 2010). The first receptor to be cloned belonged to a steroid hormone. After this, a number of techniques such as crystallography and targeted disruption of receptor genes in mice have led to the discovery of a number of nuclear receptors involved in various functions in the body (Aranda and Pascual, 2001).

Nuclear receptors can be broadly classified into two types based on their mechanism of activation. Type I nuclear receptors face the cytoplasm and are anchored by means of chaperone proteins. They are released from the chaperone proteins and enter the nucleus upon ligand binding. Examples include androgen, estrogen and progesterone receptor (Sever and Glass, 2013). Type II receptors face the nucleus and are bound to co-repressors. Upon ligand binding, the co-repressors are replaced by co-activators and transcription is regulated. Examples include thyroid hormone receptor and retinoic acid receptor (Sever and Glass, 2013). All the nuclear receptors belong to a large superfamily and they are thought to have common evolutionary origins. There are a total of about 60 nuclear receptors that have been identified so far. They differ from all other transcription factors in their ability to bind small hydrophobic molecules (Germain et al., 2003). These receptors are divided into six subfamilies or classes (Aranda and Pascual, 2001). This classification is done on the basis of multiple alignments and phylogenetic tree reconstructions. Thus, all receptors that are phylogenetically closest to one another are placed in one subfamily and each individual receptor within a subfamily is numbered (Ottow and Weinmann, 2008). A brief description of each of the subfamilies is given below (Escriva et al., 2004).

Figure 1: Classification of the nuclear receptor superfamily with examples.

1. Class I – This is a large subfamily comprising of receptors like thyroid hormone receptor, vitamin D receptor, ecdysone receptor, retinoid acid receptor, and several orphan receptors.
2. Class II – This subfamily contains receptors like retinoid X receptor and hepatocyte nuclear factor 4.
3. Class III – This is the steroid receptor subfamily comprising of receptors like progesterone receptors, androgen receptor and other orphan receptors.
4. Class IV – This is a subfamily that contains receptors like the nerve growth factor inducible factor I-B (NGFI-B).
5. Class V – This subfamily comprises of receptors like steroidogenic factor 1 (SF1).
6. Class VI – This is a small subfamily that has only one member, germ cell nuclear factor 1 receptor (GCNF1 receptor).

As described earlier, every nuclear receptor has two important domains - the DNA binding domain (DBD) and the ligand binding domain (LBD). The DBD is situated at the N-terminal of the receptor and the LBD lies at the C-terminal. The DBD is made of two zinc-binding motifs comprising of four strategically placed cysteine molecules. The LBD is made of 12 alpha helix structures for binding a hydrophobic ligand molecule (Sladek, 2010). Apart from these, each nuclear receptor also has a variable NH_2-terminal region, a linker region and a COOH-terminal region (Tian et al., 2006). Some receptors may also have one or two other regions for transcription regulation (Aranda and Pascual, 2001). The detailed structure and function of each of these regions is described below (Germain et al., 2003).

N-terminal (A/B) region	**DNA binding domain (region C)**	**Linker region (region D)**	**Ligand binding domain (region E)**	**C-terminal (F) region**

Figure 2: Backbone structure of a nuclear receptor molecule with 5 conserved regions.

- N-terminal (A/B) region – This region plays a role in transcription activation and can bind to another DNA segment to initiate transcription. It is also a site for several posttranslational modifications such as phosphorylation. It is very weakly conserved across nuclear receptor families and can vary from 23 to 550 amino acids in length.
- DNA binding domain (region C) – This is a highly conserved domain which confers DNA-ligand binding specificity to the receptor. It bears two zinc binding motifs at its N-terminal and C-terminal ends for binding zinc ions. It bears three highly conserved regions known as the P box, the D box, the T box and the A box. The functions of these boxes include conferring specificity to response elements, forming an interface for dimerization, and making contact with the DNA backbone.
- Linker or hinge region (region D) – This region is weakly conserved and acts as a hinge between the DNA binding and the ligand binding domains. It allows the receptor to undergo conformational changes without leading to stearic hindrance. It also bears a nuclear localization signal (NLS) which allows the receptor to function in the nucleus.
- Ligand binding domain (region E) – This domain confers the ligand-specific activation function to the receptor. It bears an interface for dimerization and also a repression function.
- C-terminal region (region F) – This region is weakly conserved and might have roles in coactivator recruitment to the receptor to allow specific ligand binding.

The main function of the nuclear receptor is transcription regulation and this is conferred by the DBD and the LBD (Vaisanen et al., 2011). When a ligand binds the LBD, several co-regulatory molecules are recruited to the binding site. These molecules bind different parts of the DNA sequence pulling them together and modifying the structure of chromatin. This enables the various transcription sequences to come together and specific DNA response elements to bind the DBD. This entire cascade triggered by ligand binding leads to regulation of transcription of specific genes (Sladek, 2010). Nuclear receptors recognize a specific DNA sequence – (A/G)GGTCA upstream of genes that are regulated by these receptors. This sequence is known as a hormone response element (HRE) (Harvey and Williams, 2002). It is usually present in two copies and may be arranged as palindromes, inverted palindromes or direct repeats. When the DNA binding domain of the receptors come in contact with these HREs, they lead to the cascade of events ultimately leading to transcription regulation (Laudet, 1997).

The main physiological functions of nuclear receptors include homeostasis, control of embryonic development, cell differentiation and organ physiology (Duong and Rochette-Egly, 2011). Apart from these, they also play a role in certain pathological conditions such as cancer, rheumatoid arthritis, diabetes, asthma and other hormone-resistance syndromes (Ottow and Weinmann, 2008). The ligands of these receptors belong to a wide range of signaling pathways and include steroid hormones, vitamins and metabolic intermediates (Germain et al., 2003). Nuclear receptors work by acting as mediators of various intracellular and extracellular signals involved in the regulation of gene expression. They play a role as transcription factors by binding transcriptional regulators such as hormones and metabolites (Contro et al., 2015). They act as intermediaries of different signaling pathways by undergoing various posttranslational modifications. They also play a role in signal transduction crosstalk (Germain et al., 2003). All these functions help the nuclear receptors play an active role in embryogenesis, reproduction, homeostasis, cell growth and cell death. An example is the retinoic acid receptor and the retinoid X receptor which play a role in long-term memory processes (Germain et al., 2003).

Any mutation or alteration in the sequence of the nuclear receptor leads to several human diseases. Mutations in thyroid receptor β is known to cause thyroid hormone resistance and decreased activity of thyroid hormone (Isik et al., 2013). Mutations in retinoic acid receptors have been associated with acute promyelocytic leukemia (Ozpolat, 2008). Other metabolic diseases that are linked to alterations in nuclear receptor sequence include obesity, diabetes, atherosclerosis, dyslipidemia, and uncontrolled inflammation (Germain et al., 2003). Most nuclear receptors have come to light as drug targets when scientists have used tissue extracts to treat various conditions. For example, the glucocorticoid receptor was identified when adrenal gland extracts were used for the treatment of Addison's disease (Ottow and Weinmann, 2008). The progesterone receptor was one of the first nuclear receptors to be identified as a drug target (Ottow and Weinmann, 2008). Other therapies include endocrine therapies and hormone replacement therapies (Germain et al., 2003). There are several approaches that are currently being explored with regards to using nuclear receptors for therapy. It is now possible to use drugs that can target only selective receptors and/or agonists, antagonists and ligands (Kojetin and Burris, 2013). Several nuclear receptor-independent ligand functions are also being analyzed and several factors that have an indirect effect on nuclear receptor signaling pathways are being considered for therapeutic purposes (Maggi, 2011). One example is Tamoxifen which was the first selective estrogen receptor modulator to be used for the treatment of breast cancer (Gronemeyer et al., 2004). Another example is Raloxifene belonging to the same category as above and used for protection of bones and antiproliferative effects (Gronemeyer et al., 2004).

In conclusion, the nuclear receptor superfamily is a large family comprising of very important mediators for upregulation or downregulation of gene expression. Their absence or alteration leads to deadly human diseases including cancer and diabetes. As a result, they are also very important therapeutic targets for battling these diseases. There is considerable ongoing research involving nuclear receptors and their manipulation to make way for the therapy of various human disorders.

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