NRN Nuclear Receptor News

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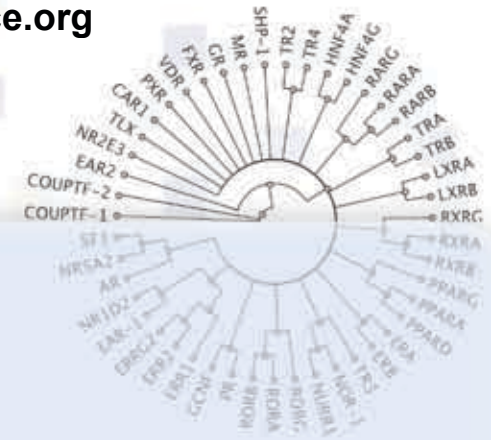

- Welcome to the Constitutive Androstane Receptor (CAR) Issue
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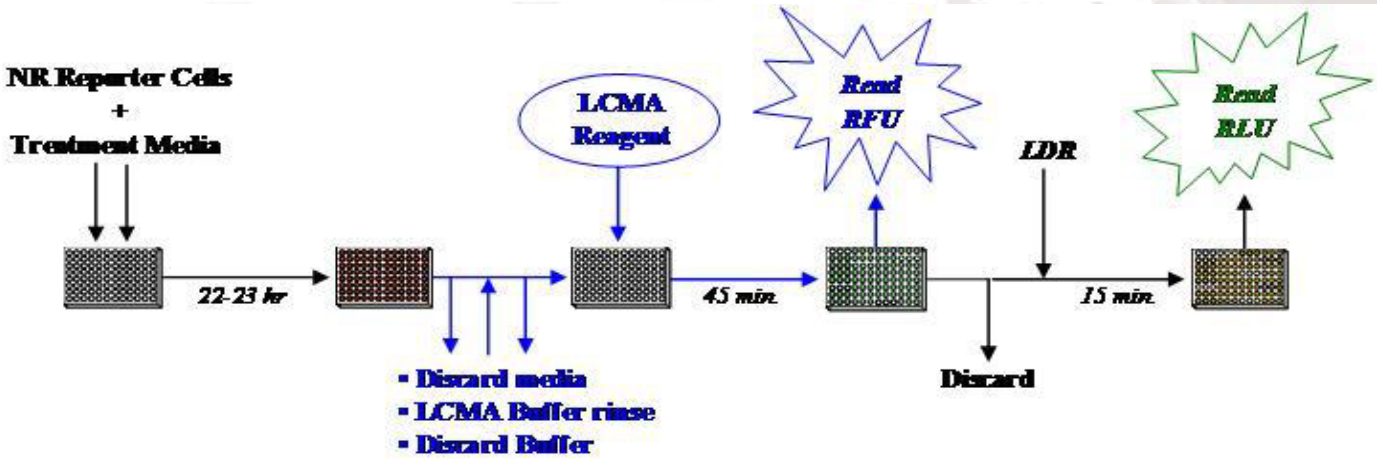
INDIGO Unveils New Live Cell Muliplex (LCM) Assay

INDIGO Biosciences, The Nuclear Receptor Company, introduces a new tool designed to help researchers gather more data when screening compounds using the INDIGO Nuclear Receptor Assay kits. The INDIGO Live Cell Multiplex (LCM) Assay kit is designed to quantify live reporter cells in each assay well, and is specifically optimized to interface with all INDIGO Nuclear Receptor assays in a multiplex protocol. The INDIGO LCMA helps to validate primary data, eliminating false positives and false negatives by determining if a compound is mitogenic or cytotoxic. The INDIGO LCM Assay is quick and convenient, and is available in single or multi-plate bulk kits.



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Nuclear Receptor News

Welcome to the Constitutive Androstane Receptor (CAR) Issue

Pregnane X receptor (PXR) and constitutive androstane receptor (CAR) are transcription factors that control the expression of a broad array of genes involved in the transport and biotransformation of many drugs and endogenous substances, such as bile acid, bilirubin, and certain vitamins. There is increasing interest in the roles of PXR and CAR in various physiological processes such as lipid metabolism, glucose homeostasis, and inflammation. Both these xenoreceptors are noted for being able to respond to a wide variety of chemicals, often in a species-specific manner. A nuance of CAR activation, in humans at least, is that the gene has multiple splice variants which result in different ligand binding domains. The species and splice variant differences in CAR activation is the focus of this issue of NRN. Please visit the Nuclear Receptor Resource (nrresource.org) to find out more information on CAR and other nuclear receptors.

Constitutive Androstane Receptor (CAR): Impact of Splice Variants

The human constitutive androstane receptor (CAR, NR1I3) regulates the expression of genes involved in xenobiotic metabolism and transport in the liver, including CYP2B and 3A4, UGT1 and MDR. Studies from mouse models show this nuclear receptor is also involved in bile acid, thyroid hormone and HDL homeostasis1. The CAR gene uses multiple alternative splicing events during pre-mRNA processing, thereby enhancing the CAR transcriptome2. The 348 amino acid long wild-type human CAR (hCAR1) is encoded by 9 exons comprised of a DNA binding domain (DBD), a hinge region, and a ligand binding domain (LBD). hCAR2 contains an additional four amino acids (VSPT) while the predominant variant expressed in the liver, CAR3, contains an additional five amino acids (APYLT)1; these insertions are found in the ligand binding domain of the receptor. The rat and the mouse CAR sequences are more similar to that of hCAR1 and there is little evidence to support the existence of splice variants in these species. The hCAR2 and hCAR3 transcripts are prominently expressed in human liver and primary hepatocytes, with combined levels ranging up to ~50% of total CAR3. Both CAR2 and CAR3 activate reporters containing response elements derived from the endogenous promoters of the CYP2B6 and CYP3A4 genes1. CAR1 is active in the absence of ligand with the unique capability to be further regulated by activators, mainly via inverse agonism 4. A number of CAR activators, including phenobarbital, do not directly bind to the receptor but affect signaling pathways that impinge on the NR's activity. Unlike CAR1, CAR3 functions as a ligand-dependent receptor 1, activating transcription in the presence of the human CAR ligand 6-(4-chlorophenyl)imi-dazo[2,1-b] thiazole-5-carbaldehyde O-3,4-dichloroben-zyl)oxime (CITCO)(See Figure 1). CAR2 has a ligand binding profile that is distinct from both CAR1 and CAR3 5, as evidenced by activation with di-ethylhexyl phthalate (DEHP).

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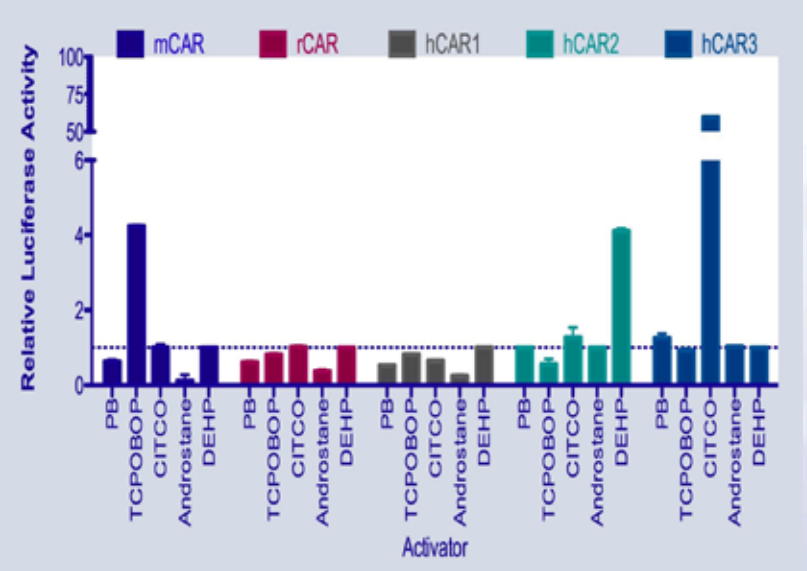

Figure 1. Activation of human, mouse, and rat CAR by reference compounds

In addition to splice variant differences in activation profiles, species differences are seen, as shown with the mouse specific CAR agonist 1,4-bis[2-(3,5-dichloropyridyloxy)] benzene (TCPOBOP). The existence of splice variants in human CAR and the species differences in activation by xenobiotics certainly makes examining this receptor a challenge. However, given the importance of CAR and other xeno-sensing NRs such as PXR in predicting drug-drug and drug-nutrient interactions, it is a endeavor worth undertaking.

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2. Auerbach, S. S., Ramsden, R., Stoner, M. A., Verlinde, C., et al. Alternatively spliced isoforms of the human constitutive androstane receptor. Nucleic Acids Res 31, 3194-3207 (2003).

3. DeKeyser, J. G., Stagliano, M. C., Auerbach, S. S., Prabhu, K. S., et al. Di(2-ethylhexyl) phthalate is a highly potent agonist for the human constitutive androstane receptor splice variant CAR2. Mol Pharmacol 75, 1005-1013 (2009).

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5. Auerbach, S. S., Dekeyser, J. G., Stoner, M. A. & Omiecinski, C. J. CAR2 displays unique ligand binding and RXRalpha heterodimerization characteristics. Drug Metab Dispos 35, 428-439 (2007).