Xenobiotic Receptors, A Journey of Rewards

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Running Title: Xenobiotic receptors

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Number of Text Pages: 13
Number of Tables: 0
Number of Figures: 0
Number of References: 22
Number of Words in Abstract: 58
Number of Words in Introduction: N/A
Number of Words in Discussion: N/A

List of Nonstandard Abbreviations:
BXR - xenopus benzoate X receptors
CAR - constitutive androstane receptor
PXR - pregnane X receptor
SXR – steroid and xenobiotic receptor
Abstract

The xenobiotic nuclear receptors pregnane X receptor (PXR) and constitutive androstane receptor (CAR) were discovered or characterized in 1998. PXR and CAR have since been defined as master regulators of xenobiotic responses through their transcriptional regulation of drug-metabolizing enzymes and transporters. This article aims to provide an overview on the discovery of PXR and CAR as xenobiotic receptors.

Significant Statement

The xenobiotic receptors PXR and CAR play a key role in drug metabolism and disposition through their regulation of drug-metabolizing enzymes and transporters.
I want to thank Drug Metabolism and Disposition (DMD) and its Mini Review Editors, Drs. Xiaobo Zhong and Yurong Lai, for planning a Special Section of Mini-Reviews on the topic of “Xenobiotic receptors” in 2023 to celebrate the 50th anniversary of DMD. This Special Section of Mini-Reviews includes an introductory Mini-Review from me, as well as those from several colleagues in the field: Bruce Blumberg, Taosheng Chen, Sridhar Mani, Hongbing Wang, and Jinhan He.

The writing of this introductory Mini-Review gave me a unique opportunity to reflect on the history of the discovery of xenobiotic receptors and my own contributions to the field of drug metabolism and disposition with one of the milestones being named the recipient of the American Society for Pharmacology and Experimental Therapeutics (ASPET) Division for Drug Metabolism and Disposition (DMDD)’s Richard Okita Award in Drug Metabolism and Disposition in 2008.

I joined the field by accident. After graduating from Beijing Medical University (now Peking University Health Science Center) in 1991 and working in a research lab for two years with my initial exposure to molecular biology, I came to the United States to pursue my graduate studies at the University of Alabama at Birmingham in 1993. Under the tutelage of the late professor Jeff Kudlow, M.D., my graduate work focused on the oncogenic roles of EGFR/ErbB2 in the skin and mammary gland using transgenic approaches, with a proud achievement of using the tetracycline inducible transgenic mouse system that was rather advanced in 1990s. Upon graduation, I applied for postdoctoral fellowships, and after a series of twists and turns, I was happy to be accepted into the laboratory of Ron Evans at the Salk Institute.
I joined the Evans lab in April 1998, when the cloning of the mouse PXR was just published by Steve Kliewer, then at Glaxo Wellcome in the North Carolina Research Triangle Park (Kliewer et al., 1998). When I first arrived at the Evans lab, I was watching Bruce Blumberg revising his steroid and xenobiotic receptor (SXR) paper, which was soon published. By studying the two papers and seeping through a series of correspondences related to Bruce’s SXR paper submissions, I learned that the Kliewer lab discovered the mouse PXR by hybridization cloning using a gene fragment in the Mouse Expressed-Sequence Tag (EST) Database and a mouse liver cDNA library. Steve named it PXR based on its activation by the pregnanes 21-carbon steroids (Kliewer et al., 1998). The Evans lab cloned the human PXR as a homolog of the Xenopus benzoate X receptors (BXR) from a human genomic library/liver cDNA library hybridized with a full-length cDNA encoding the Xenopus BXR (Blumberg et al., 1998a). BXR was originally discovered in a screen for maternally expressed nuclear hormone receptors and was cloned from a Xenopus egg cDNA library (Blumberg et al., 1998b). The human PXR was originally named by the Evans lab as SXR due to its activation by multiple natural and synthetic steroids as well as xenobiotics (Blumberg et al., 1998a). PXR became a more commonly adopted name around 2002, the year I established my independent lab at the University of Pittsburgh.

I always regard Bruce Blumberg and Steve Kliewer as my scientific heroes, because PXR is one of the last groups of nuclear receptors cloned, and the next logical step is the in vivo characterization of PXR. I remember vividly that I got the cDNA of mouse PXR from Bruce who was leaving for his independent faculty position at the University of California at Irvine, and I started to screen the mouse genomic library to construct the PXR gene targeting vector. In
addition to generating the PXR knockout mice and by talking with Ron, we came up with the idea of generating humanized PXR mice and transgenic mice harboring the constitutively activated PXR receptors. My paper that included all three mouse models was published in 2000 (Xie et al., 2000a), which essentially set the tone for my career development. An independent line of PXR knockout mice was reported by the Kliewer lab (Staudinger et al., 2001). Two other significant papers I published in Evans’s lab included a paper describing the crosstalk between PXR and CAR in xenobiotic regulation (Xie et al., 2000b), and another paper establishing PXR as a bile acid sensor that plays a role in the detoxification of cholestatic bile acids (Xie et al., 2001). The role of PXR as a bile acid sensor was independent reported by the Kliewer lab (Staudinger et al., 2001). The efficiency of my postdoctoral training benefited greatly from my graduate training. Most importantly, I was able to gain the trust of my mentors and worked very independently as a graduate student and postdoctoral fellow.

The cloning and characterization of PXR as a xenobiotic receptor benefited greatly from earlier work published by Phil Guzelian’s laboratory at the University of Colorado (Quattrochi et al., 1995; Barwick et al., 1996). The “cellular factor” and “DNA element” suggested by Phil to be responsible for the drug responsive regulation of CYP3A genes in hepatocytes turned out to be “PXR” and “PXR response element”. Phil Guzelian and his associate, Joyce Barwick, were my collaborators in the first two PXR papers that I published in 2000.

In addition to PXR, an equally exciting xenobiotic receptor is the constitutive androstane receptor (CAR), whose studies were pioneered in the laboratories of David Moore, then at the
Baylor College of Medicine (Baes et al., 1994; Wei et al., 2000), and Masa Negishi at the National Institute of Environmental Health Sciences (NIEHS) (Honkakoski et al., 1998).

My four years of working as a postdoctoral fellow in the laboratory of Ron Evans were the most satisfying and rewarding experience in my scientific career. Not only was I doing some of the most exciting research, but they also gave me opportunities to meet and interact with great colleagues. In addition to Bruce, I met Taosheng Chen and Sridar Mani while attending the then popular Keystone Nuclear Receptor Symposium in the late 1990s and early 2000s. Taosheng soon left Bristol Myers Squibb (BMS) and took his faculty position at St Jude Children’s Research Hospital and has since run a successful research program with a major focus of PXR, including the development and characterization of PXR antagonists (Lin et al., 2017). Sridar has remained at Albert Einstein College of Medicine for many years, and he has made outstanding contributions in the intestinal and immunological functions of PXR (Venkatesh et al., 2014).

I met Hongbing Wang in 2005 at an International Society for the Study of Xenobiotics (ISSX) meeting in Maui, HI. Hongbing’s work has been largely focused on CAR, and he soon relocated his laboratory from UNC Chapel Hill to the University of Maryland School of Pharmacy in Baltimore. Hongbing’s most outstanding contribution is understanding the xenobiotic functions of CAR, with a particular emphasis on the function of human CAR and human relevant drug metabolism and disposition (Stern et al., 2022a). Examples of Hongbing’s recent work are the role of human CAR in the pathogenesis of liver cancer and in the chemotherapy of breast cancer (Li et al., 2022; Stern et al., 2022b).
In reflecting on my career, a rewarding and satisfying experience is the training of next
generation of scientists. In the past 20 years, I have had the privilege of working with a large
number of graduate students, postdoctoral fellows, and visiting scholars from many countries.
Many of my trainees went on to have their successful careers in academia, industry, or clinical
medicine. I enjoy working with young people, and I feel I learn from my trainees as much as
they learn from me. The research directions in my lab have been dynamic and many of the new
directions were spearheaded by my trainees. Among examples are the roles of xenobiotic
receptors in energy metabolism and the crosstalk between drug metabolism and energy
metabolism (Gao and Xie, 2010); the mutual effects between drug metabolism and diseases
(Chai et al., 2015); the endobiotic function of xenobiotic receptors and xenobiotic enzymes in
liver fibrosis (Yan et al., 2019); and most recently, the covalent protein modifications in
oxidative liver injury (Xi et al., 2021; Xu et al., 2022). Jinhan He from Sichuan University,
another contributor of this Mini-Review series, is a successful example of my mentoring. Jinhan
spent a few years as a postdoctoral fellow and a junior research faculty in my lab before
returning to China, and he has since supervised a very successful laboratory in China.

My success in the field, including my award of the Richard Okita Award in Drug Metabolism
and Disposition (formerly known as the ASPET Division for Drug Metabolism Early Career
Achievement Award), was impossible without the support from colleagues in the field including
many senior colleagues who led the nomination and wrote letters of support. I am grateful for
the support I received and have always been conscientious to return the favors to my colleagues
and to the field. I truly believe the success and continued thriving of a research field take a
village, so we should support each other and take joy in the success of colleagues including the
junior colleagues in the field. Several actions I took to promote the field include organizing a xenobiotic receptor Thematic Issues for several journals, editing two books, and serving as associate editor, editorial board member, and *ad hoc* reviewer for many scientific journal and study sections in the field. I also have had the privilege of leading the nominations of several colleagues for the ASPET and ISSX awards.

I am confident that the field of xenobiotic receptors will continue to thrive in years to come.
Authorship Contributions

Wrote the manuscript: W. Xie.
References


pirfenidone inhibits liver fibrosis by targeting the small oxidoreductase glutaredoxin-1. *Sci Adv* 7:eabg9241.


Footnotes

In addition to colleagues whose names were mentioned in the article, I want to thank Drs. Leaf Huang, Patricia Kroboth, and Arthur Levine for recruiting me to the University of Pittsburgh as a faculty member. I also want to thank Dr. John Chiang at the Northeastern Ohio Medical University for his continuous support and mentorship. My independent research at the University of Pittsburgh was supported in part by NIH grants ES012479, CA107011, DK076962, ES014626, ES019629, HD073070, DK099232, ES023438, DK083952, DK117370, and ES030429. I am also grateful to be supported by the Joseph Koslow Endowed Professorship provided by the University of Pittsburgh School of Pharmacy.

No author has an actual or perceived conflict of interest with the contents of this article.