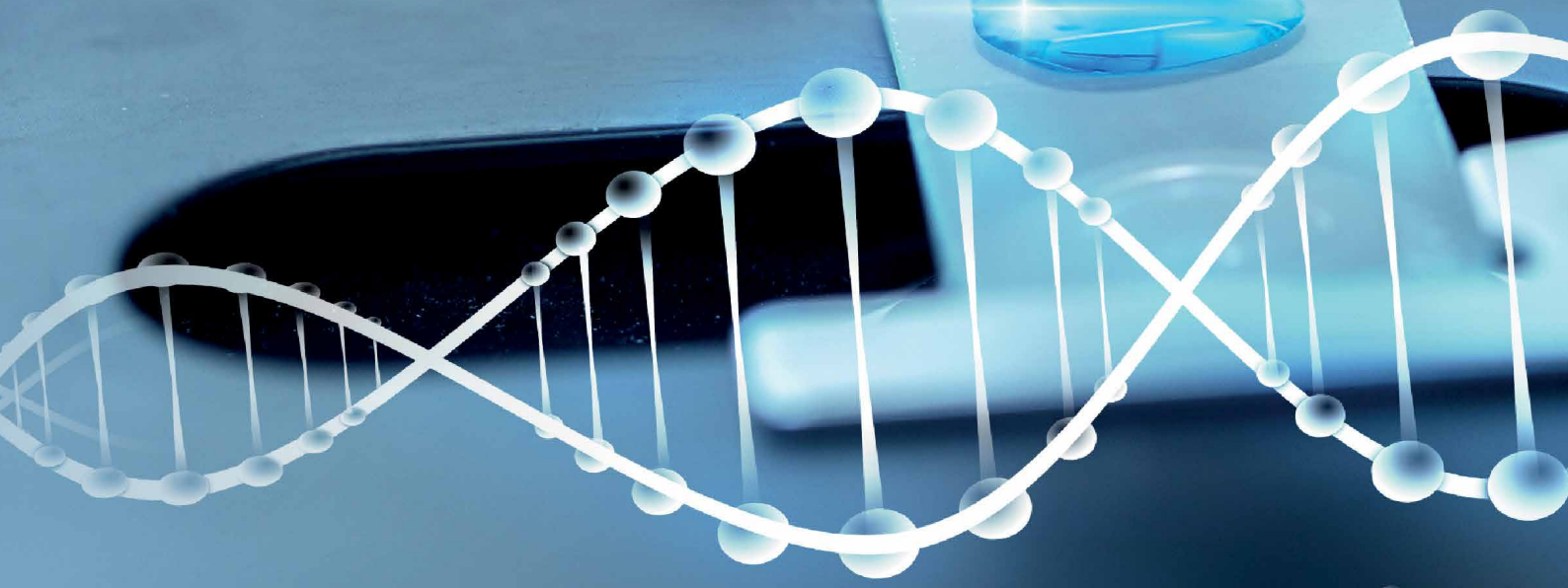
The Holy Grail of Safer Opioids: Targeting Mu Opioid Receptor Splice Variants

Dr Ying-Xian Pan



Scientia

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Despite their numerous side effects, opioid drugs and morphine-like agents have remained a pillar in the medical management of pain. Most clinically used opioid drugs act through mu opioid receptors. **Dr Ying-Xian Pan** and his team from the Rutgers New Jersey Medical School, USA, studies the molecular and cellular mechanisms of mu opioid receptors and aim to develop novel strategies and opioid analgesics for better treating pain without side effects associated with traditional opiates. Efforts to find substitutes for traditional opioid drugs are helping address the opiate abuse crisis that affects many countries around the globe.

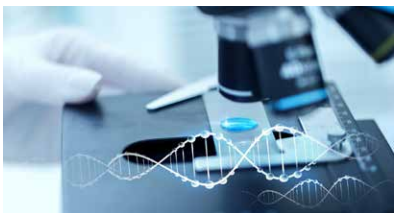
Identifying Multiple Splice Variants of Mu Opioid Receptor Gene

The actions of clinically used opioid drugs are complicated. Although potent in relieving pain, these drugs produce many side effects and their misuse has led to the global opioid epidemic. Dr Ying-Xian Pan, Professor of Anesthesiology at Rutgers New Jersey Medical School, USA, and his team have long investigated the molecular and cellular mechanisms of mu opioid actions that are mediated through mu opioid receptors. This work is providing the foundations to develop novel opioid analgesics that are devoid of unwanted side effects and the potential for abuse.

One of the main findings from Dr Pan's laboratory is that the gene encoding mu opioid receptors, known as OPRM1, is able to generate multiple isoforms or variants of the receptor through 'alternative splicing', a process that allows a single gene to produce more than one protein. Over the past years, Dr Pan's laboratory and others have identified over 65 mu opioid receptor variants from the mouse, rat and human OPRM1 gene. These variants can be categorised into three structurally distinct classes.

The first class of the variants have identical full-length seven transmembrane domains (7TM) sequences, a typical G protein coupled receptor structure, except for an alternative intracellular carboxyl terminal sequence. The second class of the variants are called truncated 6TM variants due to the lack of the first TM. The third class of the variants are truncated single TM variants. Single TM variants only contain the first TM.

Importantly, this 'alternative splicing' of the OPRM1 gene is conserved from rodents to humans, meaning that it is evolutionarily important in its functions. To define the molecular mechanisms of various analgesic drugs acting on the mu opioid receptor splice variants, Dr Pan and his team adopt multidisciplinary *in vitro* and *in vivo* approaches, including molecular biology, biochemistry, pharmacology, and behavioural studies, as well as gene targeting animal models.



Targeting Truncated 6TM variants

Dr Pan and his collaborators have long been involved in researching the Holy Grail of mu opioid analgesics. *In vivo* studies using a genetically engineered exon 11 knockout mouse model generated in Dr Pan's laboratory led to the discovery of the functional

importance of the truncated 6TM variants. In 2009, Dr Pan and his team published an article in the prestigious *Proceedings of the National Academy of Sciences of the United States of America* (PNAS), exploring the role of truncated 6TM variants on the action of heroin, a mu receptor agonist. Disrupting the exon 11 sequence that deleted all 6TM variants in the exon 11 knockout mouse did not affect the pain-relieving action of morphine and methadone. However, it attenuated the pain-relieving action of heroin.

These findings established a role for the exon 11-associated 6TM variants in heroin action and complemented the earlier finding in an exon 1 knockout mouse generated by Dr John Pintar's laboratory, in which all 7TM variants were lost but all 6TM variants were retained. Heroin analgesia was still active while morphine and methadone analgesia were completely lost.

Dr Pan and his collaborators, including Drs Pasternak and Majumdar, further extended their studies to the action of novel opioid analgesic drugs targeting the 6TM variants. In these studies, the researchers identified a novel potent opiate analgesic and published the findings in PNAS in 2011. The novel agonist, 3-iodobenzoyl-6ß-naltrexamide (IBNtxA) developed by Drs Majumdar and Pasternak, acts through the truncated 6TM variants of the murine mu opioid receptor gene (Oprm1). They observed that the analgesic action of IBNtxA was lost in the exon 11 knockout mice, indicating that exon 11-associated 6TM variants mediate IBNtxA analgesic action.

The most significant finding in the 2011 study was that despite its potent pain-relieving action, IBNtxA lacked the traditional opiate side effects, such as respiratory depression, reward behaviour, significant constipation and physical dependence. To further confirm the role of the 6TM variants in IBNtxA analgesic action, Dr Pan and his team used a gain-of-function approach to see if IBNtxA analgesia can be regained by re-expressing the 6TM variants in a complete Oprm1 knockout mouse, in which all the analgesic actions of mu opioids and IBNtxA were lost. They found that IBNtxA, but not morphine analgesia, was rescued by the lentivirus expressing the 6TM variants in a complete Oprm1 knockout mouse and published the finding in 2015 in the *Journal of Clinical Investigation* and 2018 in Anesthesiology & Analgesia.

Together, these studies demonstrated that truncated 6TM variants can be physiologically and pharmacologically important and act as new therapeutic targets for a novel class of opiate compounds displaying a vastly improved pharmacological profile.

Targeting Specific Carboxylic End of the OPRM1 7TM Variants

While the relevance of the truncated variants has been extensively explored and validated by Dr Pan and others, as described in the examples above, they examined the pharmacological roles of the full-length 7TM variants. Alternative splicing of the mu opioid receptor gene OPRM1 creates multiple full-length 7TM mu receptor variants or isoforms, which only differ in the intracellular carboxylic terminal (C-terminal) portion of the receptor. Many *in vitro* cell line studies conducted by Dr Pan's laboratory and others indicated that although these

full-length 7TM C-terminal variants bound mu opioids equally well because they shared the same binding pocket, they showed marked differences in mu agonist-induced cellular responses.

However, the main question remained regarding their *in vivo* roles in mu opioid actions, which led Dr Pan and his team to generate several C-terminal truncation mouse models to investigate the *in vivo* action of mu opioids. They created three mouse models with truncation of either all the C-termini (mE3M mice), or exon 4-encoded C-termini (mE4M) or exon 7-encoded C-termini (mE7M), and observed divergent roles for the carboxylic termini in morphine-induced behaviours in these mouse models, highlighting the importance of C-terminal variants in the modulation of complex morphine actions.

The results of the investigation, published in 2017 in the Journal of Clinical Investigation, showed that the exon 7 truncation in mE7M-B6 mice diminished morphine tolerance and reward without altering physical dependence, whereas the exon 4 truncation in mE4M-B6 mice facilitated morphine tolerance and reduced morphine dependence with no effect on morphine reward.

It is important to understand that dependence on a drug is different from drug tolerance. The latter occurs when the body needs a higher dose of the same drug to reach the same level of perceived benefits. Dependence, on the other hand, happen when the body experiences a loss of function in the absence of a drug, which can manifest itself as a series of painful withdrawal symptoms. Although tolerance and dependence often go hand-in-hand, they can happen through different biochemical mechanisms, as Dr Pan and his collaborators showed in a 2017 paper.

This paper represented a major advance in the understanding of the functional relevance of mu opioid receptor C-terminal splice variants in mu opioid pharmacology, with several important therapeutic implications. First, the researchers demonstrated that different sequences of the carboxylic terminus can alter drug-induced side effects without affecting the pain-relieving properties of opioids. Second, they showed that targeting specific carboxylic terminus of the mu opioid receptors could be an effective therapeutic strategy in the pursuit of novel drugs with more desirable pharmacological profiles and decreased side effects. Also, it is conceivable that opioid therapies targeting exon 7-associated mu receptor splice variants could decrease the addictive effects of opioids, with important implications for the opioid abuse that affects many countries.

Biased Signalling of Mu Opioids at Multiple OPRM1 Carboxylic Terminal Variants

The concept of biased signalling can be explained by looking at the large body of evidence in the medical literature showing that different agonists can trigger divergent signalling pathways through a single receptor. The co-existence of multiple OPRM1 full-length carboxyl terminal variants raises important questions about the role of these variants on the biased signalling through a single mu agonist.

In the same 2017 paper and a later 2020 paper, Dr Pan and his team demonstrated that a single mu agonist can induce biased signalling through multiple 7TM carboxyl terminal variants in terms of G protein activation and ß-arrestin 2 recruitment, providing a new perspective on biased signalling.

In particular, exon 7-associated 7TM variant, mMOR-1O, displayed greater ß-arrestin 2 bias for most mu agonists than exon 4-associated 7TM variant, mMOR-1, which explained, at least in part, why the similar morphine actions existed between mE7M-B6 and ß-arrestin 2 knockout mice. These studies opened up a new interesting research avenue for the exploration of the roles of other carboxyl-terminal sequences in biased signalling, as there are 22 full-length carboxyl-terminal variants in the mouse OPRM1 gene, 12 in the rat OPRM1 gene and 11 in the human OPRM1 gene that we know of.

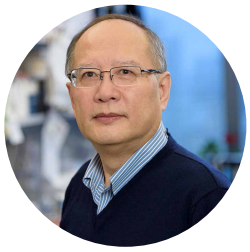
Future Perspectives

Dr Pan and his team will continue to investigate the mechanisms and functions of different mu opioid receptor variants in the action of opioids using several cutting-edge technologies. The proposed studies include mapping mu agonist induced receptor protein interactions for OPRM1 splice variants using proximity biotinylation coupled with proteomics, defining molecular mechanisms of morphine actions using phosphoproteomics and RNA sequencing approaches, and exploring molecular mechanisms and functions of OPRM1 alternative splicing using mini-gene constructs and high-throughput siRNA screening. They believe that these studies will provide potential targets for developing novel therapeutics for the treatment of pain and drug abuse.

The team led by Dr Pan will further examine the *in vivo* role played by individual OPRM1 splice variants in the pharmacological action of mu opioids by using new genetically engineered animal models. These studies will allow the researchers to link each receptor with a particular function. Dr Pan is also aiming to improve the overall pharmacological profiles of opioids by using novel targeting strategies. Evidence in the medical literature suggests that up to 80% of addicts started with prescription drugs. Efforts to find substitutes for opioid drugs would help address the opiate abuse crisis.

Finally, Dr Pan is a co-scientific founder of Sparian Bioscience. One of the most promising drugs at Sparian Bioscience, SBS-1000, is the second generation of IBNtxA that is more potent than morphine but without the many side effects associated with traditional opiates. This compound is expected to be in a Clinical Phase I Trial in early 2022.

Meet the researcher



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Dr Ying-Xian Pan obtained his MD in 1982 from the Shanghai University of Traditional Chinese Medicine, China. In 1986, he obtained his MS Degree in Biochemistry from the Shanghai University of Traditional Chinese Medicine and eventually moved to the USA, where he obtained his PhD in Physiology and Biophysics in 1993 from the University of Cincinnati, Ohio. Dr Pan continued his research career as an Aaron Diamond Foundation Postdoctoral Fellow at the Memorial Sloan-Kettering Cancer Center, New York, where he met his mentor Dr Gavril W. Pasternak, a renowned pharmacologist. After completing his postdoctoral fellowship, he joined the faculty of the Department of Neurology in 1999, where he became a full Member in 2013. Dr Pan is currently Professor of Anaesthesiology at the Rutgers New Jersey Medical School, and a core member at the Rutgers Brain Health Institute. Through studying the mechanisms of opioid actions, Dr Pan aims to develop novel strategies and drugs for treating pain and combating the opioid epidemic. Dr Pan is a co-scientific founder at Sparian Biosciences, a company devoted to the development of safer alternatives to opiate drugs.

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FURTHER READING

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