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REVIEW ARTICLE

Targeting endothelin receptors: A promising strategy for alleviating cancer-related and non-cancer pain in Animal Models

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ABSTRACT

Endothelin is a potent vasoconstrictor known to play a role in various painful conditions. This study aims to explore the role of the endothelin system, including its receptors, isoforms, and converting enzymes, in the pathogenesis of cancerous and non-cancerous ailments. Additionally, it evaluates the efficacy of endothelin receptor antagonists in managing pain associated with these conditions. A review of recent studies was conducted to identify the fundamental activities of the endothelin system and the impact of antagonists targeting endothelin receptors on pain relief. The endothelin system has emerged as a crucial player in various painful conditions. Antagonists targeting endothelin receptors have shown promise in alleviating cancer pain, with endothelin secretion observed in cancer cells of diverse histologic types. Moreover, endothelin receptor antagonists have demonstrated potential in managing neuropathic and inflammatory pain syndromes. Therapies targeting the endothelin system, particularly endothelin receptor antagonists, hold significant promise for managing both cancer-related and non-cancer-related pain syndromes, highlighting the therapeutic potential of these agents in pain management.

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INTRODUCTION

Endothelins (ETs) are a family of peptides comprising 21 amino acids that belong to a peptide family encoded by distinct genes (Davenport *et al*., 2016; Ji *et al*., 2023). Among these peptides, ET-1, ET-2, and ET-3 are the three main variants (Angeli *et al.*, 2021). Significant resemblance exists between the ET isoforms and sarafotoxins (in snake venom), particularly at the carboxy terminus. The endogenous endothelin peptides are involved in various pain-related functions (Haryono *et al.*, 2022). Endothelin receptor antagonists can be administered locally to manage pain exacerbated by skin incisions, inflammation, cancer (Lutz *et al.*, 2018). Endothelin, which induces pain only at high concentrations, can be administered locally to mimic many of the acute effects of endogenously generated endothelin (Banecki and Dora, 2023). ET-1 is the most prominent and efficient isoform. The peptide has significant vasoconstrictors and favorable chronotropic and inotropic effects on the myocardium (Haryono *et al.*, 2022). Usual sources of ET-1 synthesis encompass a variety of cell types, including vascular smooth muscle cells, mesangial cells, endothelial cells, leukocytes, mast cells, tumor cells, and cardiomyocytes (Hans *et al.*, 2009; Haryono *et al.*, 2022). ET-1 binds to two distinct types of receptors in mammalian species: the endothelin-A receptor (ETAR) and the endothelin-B receptor (ETBR). These receptors are G-protein-linked transmembrane receptors found in both non-vascular and vascular tissues. The binding of ET-1 to these receptors elicits different physiological effects, contributing to various biological

processes in the body. Most brain cells express ETBR compared to ETAR in non-neuronal and neuronal cell types (Koyama, 2021). ET-1 can activate a wide range of G protein signaling pathways, and therefore, it is involved in different biological processes, including ion transport, vascular permeability, inflammation, and blood flow, as well as blood flow regulation and muscle contraction (Banecki and Dora, 2023). ETs and their corresponding receptors, ETAR and ETBR, are centrally involved in various aspects of tumorigenesis, angiogenesis, cell proliferation, and apoptosis (Haroun *et al.,* 2023). It is also believed that ET-1 contributes to pain pathophysiology, and both receptor subtypes take part in ET-induced nociception (Angeli *et al.,* 2021). The manuscript elaborates on the mechanism and endothelin's mode of action in the pathogenesis and treatment of various pain-related issues. The objective of this study was to investigate the role of the endothelin system encompassing its receptors, isoforms, and converting enzymes in the development of both cancerous and non-cancerous conditions. Additionally, it seeks to assess the effectiveness of endothelin receptor antagonists and related therapy in alleviating pain and associated disorders.

ET-1 origin: The ET peptide family comprises three isoforms of cyclic peptides, each consisting of 21 amino acids. These peptides are encoded by different genes (Ding *et al.,* 2020; Patel *et al.,* 2020). This family was first identified in 1989 as a unique group of peptides synthesized by the vascular endothelium (Yanagisawa *et al.,* 1988). The endothelins function has received considerable attention due to their pivotal role in the circulatory system, primarily because of their potent vaso-constrictive effects (Nappi *et al*., 2022). However, following their discovery, it became evident that ET ligands are also distributed within the central nervous system, where they exert diverse actions including neurotransmission, cell division, and proliferation (Davenport *et al.,* 2018). Indeed, ET-1 was the initial subtype identified and is found expressed across diverse tissue types, including nerve tissues. Common sources contributing to ET-1 synthesis encompass endothelial cells, vascular smooth muscle cells, leukocytes, mast cells, and tumor cells (Hans *et al.,* 2009). The transcriptional regulation of the prepro-ET-1 gene, which encodes the precursor protein, and subsequent processing post-translation are crucial in controlling ET-1 biosynthesis. The prepro-ET-1 gene, responsible for encoding the precursor protein, governs the synthesis of ET-1. The prepro-ET-1 protein undergoes cleavage by endopeptidases to produce big-ET, which is an inactive precursor. The transformation of big-ET to ET-1 is aided by a group of enzymes called endothelin-converting enzymes (ECEs). There are three different isoforms of ECEs: ECE-1, ECE-2, and ECE-3. These isoforms exhibit variations in cell distribution, substrate selectivity, and cellular localization (Davenport *et al.,* 2016). The two primary isoforms responsible for cleaving big-ET to produce ET-1 are ECE-1 and ECE-2. Both ECE-1 and ECE-2 play roles in the formation of ET-1 as well as in the breakdown of amyloid-β proteins, which are implicated as causative factors in AD (Palmer *et al.,* 2010). As a result, the involvement of ECEs in AD pathogenesis has been examined from the amyloid-β protein breakdown and the production of ET (Palmer and Love, 2011). The primary objective of endothelin research in recent decades has been the development of molecular ligands targeting both allosteric and orthosteric binding sites. Notably, several newly discovered therapeutic methods have been shown to target these endothelin receptors against various pain related disorders.

Receptors and signaling: The ETAR and ETBR receptors are two subtypes of G-protein-coupled receptors through which ETs exert their effects (Wang *et al*., 2022; Arndt *et al*., 2024). The ETAR receptor has a greater affinity for ET-1 and ET-2 compared to ET-3 among the endogenous endothelin ligands. However, all three ET ligands exhibit identical affinity for the ETBR receptor (Davenport *et al.,* 2016) . Both ET receptors raise intracellular Ca^{2+} via activating phospholipase C (PLC) and are associated with the Gq protein (Koyama, 2021; Di Maio *et al*., 2023). ETAR receptor activation is typically associated with Gs coupling, resulting in an elevation of cyclic adenosine monophosphate (cAMP) levels. Conversely, the ETBR receptor is coupled with Gi, leading to the inhibition of the signaling pathway (Di Maio *et al*., 2023). The G12/13 protein is also associated with the ETAR and ETBR receptors. Signals initiated by the G12/13 protein activate the Rho protein. This activation then leads to the subsequent activation of Rho-associated protein kinase (ROCK) (Hans *et al.,* 2009). Indeed, ET receptors, like to numerous other G-protein-coupled receptors (GPCRs), have been found to form dimers. However, it's notable that activation of either ETAR or ETBR receptor homodimers by ET-1 typically does not lead to a sustained increase in intracellular calcium levels (Evans *et al.,* 2008).

The cell bodies of small-diameter sensory neurons, particularly those situated in the dorsal root ganglia (DRGs) contain a significant proportion of ETAR. DRGs are linked with Aδ- and C-fibers that are responsible for carrying pain impulses (Pomonis *et al.,* 2001; Khodorova *et al.,* 2009; Chen *et al*., 2023). The collective data presented form a compelling argument supporting the notion of selective engagement of peripheral ETAR receptors in triggering nociceptive fiber activation and subsequent pain sensation. ET-1, through intricate receptor pathways, is implicated in pain induction. Specifically, activation of ETAR receptors can lead to elevated intracellular calcium levels in certain instances. This rise in Ca^{2+} levels within peripheral sensory neurons is attributed to ET-1, concurrently facilitating function of TRPV1 channels (located on nociceptive C-fibers). This enhancement involves (Ca^{2}+ independent) mechanisms, such as PKC-ε-mediated phosphorylation, thereby modulating pain perception (Di Maio et al., 2023). It is probable that a modulatory influence on tetrodotoxin (TTX)-resistant Na^{+} channels contribute significantly to the potentiation of TRPV1 activity. This modulation may also be influenced by PKC activity (Khodorova et al., 2009). It is uncertain which G protein subunits are mediating these effects. ETBR receptors predominantly express in dorsal root ganglion (DRG) satellite cells (Pomonis *et al.,* 2001). It is considered that prostaglandin

E2 is produced and released (compound involved in inflammatory pain).

Additionally, keratinocytes also contain ETBR receptors. These receptors cause to release the β-endorphin from keratinocytes, which in turn exerts a local analgesic effect (Khodorova *et al.*, 2003). Signaling pathways that cause this effect are still under study. Even though Gi2, Gi3, and Gs have been identified in keratinocytes (skin epidermis) (Koyama, 2021). It is believed that in these cells, adenylyl cyclase activation causes an increase in intracellular calcium through a mechanism that might cause the release of β-endorphins (Khodorova *et al.*, 2009) (Figure 1). The transient receptor potential (TRP) channels, comprising a substantial family of receptors, have been the primary focus of research concerning nociception. TRP is composed of six subfamilies: TRP vanilloid (TRPV), mucolipin (TRPML), canonical (TRPC), melastatin (TRPM), polycystin (TRPP), and ankyrin (TRPA) (Takayama *et al.*, 2019). TRPV receptors, found on C-fiber nociceptors, detect pain signals related to noxious heat and contribute to the sensation of heat hyperalgesia (Tsagareli *et al.*, 2020).

Inhibitors or intervention: Several peptides and non-peptide compounds have been identified that exert their effects on endothelin receptors with varying specificity and potency. Some of these compounds work as agonists, while others work as antagonists. Some are non-selective, while others work selectively on the endothelin receptors (Davenport *et al.*, 2016; Haryono *et al.*, 2022). Extensive research has been conducted on the development of antagonists and agonists for both endothelin receptors, ETAR and ETBR. Among the initial discoveries were FR139317 and BQ123, which were identified as the first ETAR-selective antagonists. After the discovery of ET-1, an antagonist and non-peptide drug were developed for the endothelin system, showing promising efficacy. Endothelin (ET-1, ET-2, and ET-3) are the agonists of the ETBR and ETAR receptors. As the affinity of ET-3 for the receptor ETAR is less, it may cause more activation of the ETBR receptor (Haynes *et al.*, 1995; Haryono *et al.*, 2022). No agonists for the receptor ETAR have been discovered in both the categories of peptides and non-peptides. It is widely recognized that the activation of ETAR during pathophysiological conditions results in harmful effects, and no evidence is available that the activation of ET-1/ETAR has any beneficial effects (Davenport *et al.*, 2018). Indeed, various receptor agonists for ETBR have been identified over time. For instance, Sarafotoxin 6c, utilized in human experimental studies, exhibits less selectivity for humans but demonstrates high selectivity for ETBR receptors in rats (Russell and Davenport, 1996). The most commonly used selective receptors for the ETBR are BQ3021 (Ihara *et al.*, 1992) and IRL1620 (Takai *et al.*, 1992). IRL1620 is used as neuroprotective agent evidenced in an experimental trial (Briyal *et al.*, 2019). The BQ3020 is used for the characterization of ETBR receptors and also in labeling studies (Perreault *et al.*, 1995). Additionally, it is utilized as a PET agent in *in vivo* studies (Johnström *et al.*, 2006). No evidence currently supports the idea that endothelin agonists are used in cardiology. Endothelin receptor antagonists indeed have a longer history of usage. These antagonists can be categorized as either selective, targeting one non-specific or specific receptor, or dual antagonists, which block both receptors (Table 1).

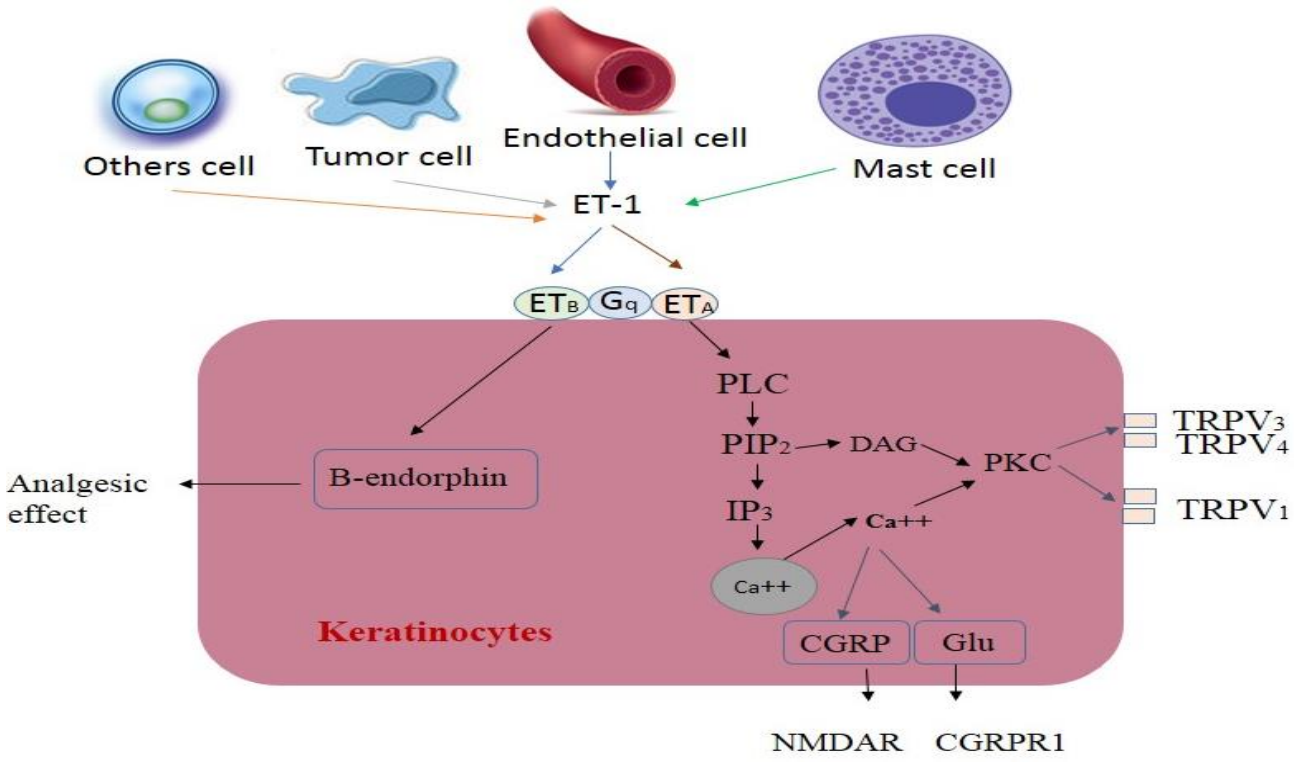

Fig. 1: A schematic diagram illustrates the signaling pathways initiated by ET-1 in keratinocytes. Endogenous synthesis and release of ET-1 are believed to activate local ETAR receptors, which in turn regulate processes such as cell proliferation and the release of noxious chemical stimuli like calcitonin gene-related peptide (CGRP) and glutamate. ETBR receptors in keratinocytes can induce acute stimulation of β-endorphin release, leading to local analgesic activity.

Table 1: Agonists and antagonists for ET receptors.

Receptor type	Antagonist	References	Agonist	References
ETAR selective	Ambrisentan, Sitaxsentan, Atrasentan, Clazosentan, Zibotentan,S-0139,SB234551,Ro-61-1790	(Vatter and Seifert, 2006; Koyama, 2021)	Sarafotoxin 6b	(Koyama, 2021; Banecki and Dora, 2023)
ETBR selective	BQ788,IRL-2500,A192621,RES-701-1	(von Geldern *et al*., 1999; Koyama, 2021; Banecki and Dora, 2023)	Sarafotoxin 6c, IRL-1620, BQ3020	(Takai *et al*., 1992; Koyama, 2021)
Non selective type	Macitentan, Bosentan	(Bolli *et al*., 2012; Bhalla *et al*., 2015; Koyama, 2021)	ET-1	(D'Orléans-Juste *et al*., 2019)

The BQ123 is the most used selective antagonist for the ETAR receptor in both *in vitro* and *in vivo* experiments. The other peptide-based selective antagonists for the ETAR receptors used in the research studies are TAK-044 (Masuda *et al.,* 1996), FR139317, Sitaxentan (Wu *et al.*, 1997), and ambrisentan (Vatter *et al.*, 2003) to treat PAH in clinical trials. Sitaxentan was withdrawn in 2010 due to reports of liver failure and mortality due to idiosyncratic hepatitis (Don *et al.*, 2012). Another selective antagonist for the ETAR receptor used for treating diabetic nephropathy is atrasentan (Jarvis *et al.*, 2000). The selective receptor antagonists for the ETBR are not well developed than that of the endothelin receptor antagonists due to their potential to cause vasodilatation effects and the blocking of ET-1 clearance (Davenport *et al.*, 2016). A monoclonal antibody against ETAR receptors (ETRQ-002vaccine/mAb) has developed in the treatment of PAH with favorable results in animal model. *In vitro* studies (diabetes animal model) have showed promising therapeutic effects of ET traps on diabetic target organs, including the kidney and heart, without side effects. As a result, The ET trap is an appealing approach for future treatment of diseases associated with ET-1.

ET-1 modulates pain: Cancer and related issues including cancer pain is the spreading problem in all over the globe (Teng *et al*., 2016; Saini *et al*., 2020; Ali *et al*., 2022; Chen *et al*., 2022; Afzal *et al*., 2023; Maaruf *et al*., 2023). Globally, it is anticipated that the number of cancer cases per year will rise from 14 million in 2012 to 22 million by 2032 (Nabal *et al.*, 2012). ET-1 contributes an important part in pain and related issues. There is no doubt that ET-1 functions as an algogen in the peripheral nervous system (PNS) and is engaged in the etiology of many pain related syndromes, including neuropathic, cancer, and inflammatory pain (Haroun *et al.*, 2023). Non-neuronal cells in the PNS, such as endothelial cells, mast cells, monocytes, and macrophages, can express and secrete endothelins (Banecki and Dora, 2023; Haroun *et al*., 2023). Due to pain related issues, pain management is the necessary need of time.

Non-cancerous pain

Inflammatory Pain: During inflammatory conditions, various chemicals are released that cause the sensitization and excitation of different nerves and cause pain (Matsuda *et al*., 2019; Verma *et al*., 2015). The secretion of ET-1 is significantly increased in inflammatory conditions. It is shown that ETBR receptor antagonists, rather than ETAR receptor antagonists, inhibit responses to nociceptor stimulation in rats with knee joint inflammation. The ETBR stimulation has a role in inflammation, and ETBR knockout mice showed lower cutaneous inflammatory responses on the topical application of arachidonic acid (Griswold *et al.*, 1999). Similarly, the infiltration of neutrophils in the ETBR knocked-out mice was lower compared to the wild type. However, numerous studies showed that ETAR activation affects the inflammatory process. For instance, it has been demonstrated that inflammatory cytokines promote the ETs production. Ultimately, it promotes the chemotactic attraction of monocytes and neutrophils (Banecki and Dora, 2023).

Furthermore, cutaneous ETARs play a role in thermal hyperalgesia during chronic inflammation induced by complete Freund's adjuvant (CFA) and acute inflammation induced by carrageenan in mice (Baamonde *et al.*, 2004). ETAR antagonist as a pre-treatment before the induction of inflammation resulted in the complete blockage of the hyperalgesia in both inflammatory conditions, and no effect was observed with an ETBR antagonist treatment (Banecki and Dora, 2023). These findings show that the ETAR promotes pain, inflammation, and hyperalgesia, and the activation of ETBR may cause the opposite actions. The development of the ischemia may depend on the inflammatory responses (Hans *et al.*, 2009). The ET-1s have been labelled as the profibrotic cytokine due to their essential role in immune responses. It modulates vascular permeability while having an important role in the immune system. The ET-1 treatment in conscious rats causes an increase in hematocrit in a dose-dependent manner (Filep *et al.*, 1991; Banecki and Dora, 2023). The ET-1 can influence the immune system through other mechanisms like transcription of different pro-inflammatory cytokines. The ET-1 may cause the increased release of various cytokines from the monocytes, like interleukin 1 and 6 (IL-1 & IL-6) and tumor necrotic factor alpha (TNF-a). Together, these cytokines promote chemotaxis, also stimulated by ET-1 (Haryono *et al.*, 2022). Interestingly, these cytokines can also stimulate the release and synthesis of ET-1 (Wood *et al.*, 1999; Haryono *et al.*, 2022). Indeed, receptor antagonists hold promise for mitigating inflammatory responses. While initially discovered in the vasculature, ET-1 exerts effects on all major organs within the body. Fluctuations in the levels of ET-1 have pathological implications throughout the body, and it is therefore associated with many diseases.

Neuropathic pain: The ET-1 modulates the nociceptive pain by influencing the neurotransmission in the ascending pathways of pain (Khodorova *et al.*, 2009). It induces hyperalgesia in the spinal cord by causing the activation of ETAR receptors. The ET-1 is also associated with the anti-nociceptive effect that is supposed to be

mediated via the ETBR receptors. The time regulation of the neurotransmitter Endothelin and ion channels causes the influence on the pain pathways (via the ET-1). The hyperexcitation of various neuronal networks in the spinal cord causes neuropathic pain. However, the precise mechanisms causing the pain pathways to become overexcited are not fully understood. Some studies showed more reactive astrocytes (spinal cord region) in experimental animals suffering from neuropathic pain (Koyama, 2021). The Stat3 inhibitor treatment in rats (suffering from neuropathic pain) results in the recovery from established hyperalgesia and suppresses the development of reactive astrocytes (Tsuda *et al.*, 2011). Reactive astrocyte induction in the spinal cord increased the astrocytes ETBR receptors in neuropathic pain caused by allergic inflammation. Astrocytes are suppressed as a result of the application of BQ788, which reduces hyperalgesia (Yamasaki *et al.*, 2016). These findings demonstrated that neuropathic pain results from the ETBR- receptors activation of reactive astrocytes.

Cancer pain

Different cancers: ET-1 is produced by a variety of cancers, such as the prostate, breast, colon, hepatocellular, pancreatic (Haroun *et al.*, 2023). However, all cancers did not produce ET-1 (Pickering *et al.*, 2008). Cancer produces endogenous ET-1, which causes mechanical hyperalgesia in a mouse model of cancer pain (Hans *et al.*, 2009). The highly selective ETAR antagonist BQ123 reduced nociceptive behavior, as assessed through the duration of paw licking. Nevertheless, systemic treatment with the ETAR antagonist and simultaneous tumor injection of an ETBR antagonist had no effect on pain behavior before ET-1 injection (Quang and Schmidt, 2010). Using another comparable sarcoma model showed neural sensitization through behavioral and electrophysiologic analysis (Cain *et al.*, 2001). On the sixth day after receiving the sarcoma vaccine, paw withdrawal hyperalgesia was seen in the animals with tumors. The development of the sarcoma increased spontaneous C-fiber activity.

The reaction threshold to heat was significantly lower in the C-fibers of the cancer-affected animals compared to control mice. Both C and A-fibers are excited by ET-1, when it is injected subcutaneously into the plantar surface of the hind paw. In the sarcoma mouse model, it was interesting to note that the A-fibers lacked spontaneous activity.

Antagonizing ETAR with BQ-123 produced antinociceptive effects comparable to those of acutely injected high doses of morphine (Schmidt *et al.*, 2007). Tumor volume promotes nociception, however ET-1 concentration seems to be a more important factor in determining nociception than tumor volume (Pickering *et al.*, 2008). The ETAR has been the central focus of the majority of prior research on cancer pain and ET-1. The ETBR role in cancer-related pain has not been thoroughly explored. In a mouse model of metastatic sarcoma, it has been demonstrated that an ETAR antagonist's treatment dramatically reduced ongoing and movement-evoked pain behavior while ETBR antagonism enhanced the pain behavior (Peters *et al.*, 2004). Animal models employed in cancer pain research alongside potential targets for analgesia are shown in the Table 2. In rats, rabbits, and monkeys, ETBR expression is observed in DRG satellite cells and non-myelinating Schwann cells within the sciatic nerve, whereas ETARs are found dispersed throughout peripheral sensory neurons (Peters *et al.*, 2004).

Keratinocytes are known to release opioids and reported to have ETBRs (Wintzen *et al.*, 1996; Koyama, 2021). Cutaneous skin injury causes to release of ET-1, which further activates the ETAR to cause pain. Keratinocytes have a well-documented role in controlling the activity of the skin's primary afferent nociceptors that surround them. One possibility is that keratinocytes could be a source of endogenous opioids released upon ETBR activation, as naloxone can block the analgesic effect arising from ET-1-mediated activation of ETBRs on keratinocytes (Khodorova *et al.*, 2003). This model provides intriguing information regarding the possible analgesic effect of ETBR activation in carcinomas, specifically in cancers of the epithelium. High levels of ET-1 are produced by oral squamous cell carcinoma, a cancer arising from oral keratinocytes (Pickering *et al.*, 2007; Schmidt *et al.*, 2007). Vasoactive potentials (vasoconstriction and vasodilation) of ET-1 may provide dual-level control of cancer pain. Activation of ETBR on malignant keratinocytes may lead to analgesia through an

Table 2: Animal models used to study cancer pain and targets for analgesia (Lindsay *et al.*, 2005; Nagamine *et al.*, 2006; Symons *et al.*, 2008; Lam *et al.*, 2012; Haroun *et al.*, 2023).

Mouse model with strain	Cancer pain model induction approach	Methods to evaluate the behavior of pain	Tested for analgesics
A pancreatic cancer model in transgenic mice	Transgenic mouse stimulated by the rat elastase-I promoter to express the large T antigen of the simian virus 40	Hunching behaviour Palpation-evoked vocalisation	Morphine sulfate (10 mg/kg, s/c)
Fisher rats for squamous cell carcinoma	SCC-158 cells were introduced into the subperiosteal tissue on the lateral aspect of the lower gingiva in rats	Dynamic plantar aesthesiometer was used for assessing paw sensitivity, with thermal sensitivity measured using a 55 ± 0.5°C hotplate for whisker-pad skin and submandibular skin, as well as radiant heat for the paw. Mechanical sensitivity was evaluated using von Frey filaments for both whisker-pad skin and submandibular skin	N/A
A neuroma-induced pain model in Sprague-Dawley rats	The tibial nerve is ligated and positioned just above the lateral malleolus, facilitating the formation of a neuroma	Von Frey test for behavior evaluation	Lidocaine injection (1% lidocaine injected subcutaneously in 100 µl with epinephrine)
Oral squamous cell carcinoma (SCC) in BALB/c mice model of pain	Injection of 50 µl of human SCC into the left lateral tongue	Dolognawmeter (operant measure)	20µg of soybean trypsin inhibitor intratumour

opioid mechanism. Conversely, activation of ETAR on neurons within the tumor microenvironment induces pain. The fact that ETAR antagonism causes anti-nociception while preventing morphine tolerance is noteworthy and may offer an efficient method of treating cancer pain.

Cancer pain management: Globally, pain management has been a challenge recently, and many existing pain treatments are ineffective or have significant adverse effects. However; some approaches may useful to manage the pain. The pain signaling pathway includes endothelin, and its receptors ET-1 itself has both nociceptive and anti-nociceptive characteristics depending on the local concentration (Piovezan *et al.,* 1997; Enevoldsen *et al.,* 2020). ET-1 has the capability to potentiate the effects of algogens, such as capsaicin and arachidonic acid (Khodorova *et al.*, 2009). In addition, ET-1 plays a part in inflammatory pain, cancer pain, diabetic neuropathy, and neuropathic pain (Furukawa *et al.,* 2018). There has been evidence of increased plasma ET-1 in animal models of many clinical diseases, such as prostate cancer, vaso-occlusive crisis, and complex pain syndrome, where pain is a key symptom (Furukawa *et al.,* 2018). Most ERA trials for the cure of pain have been carried out in animal models such as sick cell disease and cancer pain (Lutz *et al.*, 2018). There was also evidence of reduced pain in

patients having bone and prostate cancer (Enevoldsen *et al.*, 2020; Haroun *et al.*, 2023). Indeed, additional research is warranted to comprehensively elucidate the specific effects of ET-1 on nociception.

Opioids are considered effective medicine in curing pain. However, various AE s and addictive behavior of this medicine remains a problem (Williams *et al.*, 2001). Chronic opioid exposure causes a variety of cellular adaptations, including the desensitization and downregulation of pathways. Hence, in chronic pain and

addiction patients, it has been observed that administering morphine at doses 100 times higher than the standard analgesic dosage can result in mild physiological effects (Williams *et al.*, 2001). Additionally, research in animal models suggests that endothelin contribute a part in controlling the pharmacological effects of morphine and can interact with opioids (Williams *et al.,* 2001; Enevoldsen *et al.,* 2020).

ATP is highly expressed and easily produced during inflammation in malignant tissue (Wang *et al*., 2021). Purinergic receptors, which include ATP receptors, consist of P2X receptors (ligand-gated ion channels) and P2Y receptors (part of the GPCR superfamily) (Aley *et al*., 1996). A mouse model of CIBP was used to show that cutaneous hypersensitivity (as checked via von Frey test) can be decreased by inhibiting P2X3 receptors with a monoclonal antibody, although skeletal pain-like responses remain largely unaffected (Haroun *et al*., 2023).

Preclinical research on chemotherapy-induced neuropathy suggests that glutamate may offer protection against neurotoxicity caused by cisplatin or paclitaxel (Bae *et al*., 2021). This is evidenced by reduced proprioceptive loss and improved rotarod test performance in rodents during dark cycles (Nozaki-Taguchi *et al*., 2001). Additionally, treatment with anti-NGF neutralizing antibodies significantly reduces skeletal pain in animal models of cancer-induced bone pain (CIBP) by inhibiting ectopic sprouting and preserving bone integrity (Polomano *et al*., 2001; Hong *et al*., 2020).

Anti-NGF therapy has also shown promising results in non-cancer models of skeletal pain. For instance, in mice with femoral fractures, anti-NGF treatment reduces pain behaviors without hindering bone repair (Malfait *et al*., 2020). Anti-NGF neutralizing antibodies have been shown to improve cachexia and hyperalgesia in a mouse model of autoimmune arthritis, without affecting joint destruction or severe inflammation. Several NGF-binding monoclonal antibodies have been tested in many experimental trials for various chronic pain conditions including osteoarthritis (Smith *et al*., 2004). Tanezumab has been investigated as a possible painkiller for osteoarthritis, however it is not approved by FDA due to its associated side effects. (Haroun *et al*., 2023).

Moreover, many animal studies have demonstrated that antagonists targeting endothelin A receptors (ETAR) can augment the analgesic effects of morphine (Enevoldsen *et al.,* 2020) and can help morphine-tolerant mice couple their G-proteins to their receptors, where an uncoupling of G-proteins occur. Additionally, it has been noted that ETAR-selective ERAs stimulate the secretion of Leu-enkephalin and endorphin (Quang and Schmidt, 2010). It has been suggested that ETAR receptor agonists could reduce the withdrawal effects of opioids in animal studies. These findings strongly suggest the involvement of endothelin receptors in central nervous system pathways implicated in opioid withdrawal (Bhalla *et al.,* 2015). As a result, endothelin A receptor antagonists possessing favorable pharmacokinetic characteristics could potentially offer therapeutic benefits in combination therapy with opioids, addressing both opioid tolerance and withdrawal issues.

Concluding remarks: One of the main goals of pain research should be to identify the molecular mechanisms behind the diverse features of nociception. There is an urgent need for novel, mechanistic-based medicines. Several investigations have demonstrated that the ET axis significantly influences the extent of both chronic and acute pain (Banecki and Dora, 2023; Haroun *et al.*, 2023). ETs could potentially impact nociception by directly stimulating nociceptors or inducing sensitization. Elevated levels of Endothelin-1 (ET-1) may further sensitize neurons containing Endothelin A receptors (ETAR), particularly those previously activated due to tissue damage, as studies have shown that ET-1 enhances mechanical, thermal, and chemical hyperalgesia when administered externally. Administering endothelin receptor antagonists could potentially aid in pain management, both independently and in combination with other analgesic medications. Developing specific antagonists targeting the two subtypes of endothelin receptors would greatly enhance our understanding of nociception across different pain conditions. ET-1 plays a significant role in various types of pain, including inflammatory pain, cancer-related pain, diabetic neuropathy, and neuropathic pain (Khodorova *et al.,* 2009; Haroun *et al.,* 2023). Most ERA trials for the cure of pain have been carried out in animal models (Hans *et*

al., 2009; D'Orléans-Juste *et al.*, 2019; Haroun *et al*., 2023). Therapies targeting the endothelin system, particularly endothelin receptor antagonists, hold significant promise for managing both cancer-related and non-cancer-related pain syndromes, highlighting the therapeutic potential of these agents in pain management. To discover novel targets for pain relief, a deeper comprehension of the unique mechanisms underlying cancer-related pain states is imperative. Future research that investigates into the peripheral interactions between non-neuronal cells and neuronal in animal models representing various forms of cancer-related pain will be pivotal in uncovering the fundamental pathophysiological mechanisms behind cancer-related and non-cancer-related pain and their management.

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