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REVIEW PAPER

Orphan Nuclear Receptors in Colorectal Cancer

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Abstract
Colorectal cancer is one of the most common cancers worldwide, with an overall increased incidence annually. Despite improvements in treatment and surveillance, almost 50% develop recurrent and/or distant disease. Unknown cellular processes are the fundamental cause for treatment failure and metastatic disease. The interplay of chronic inflammation and carcinogenesis is well established. Recent work has highlighted the role of nuclear receptors and co-regulators in the inflammation to carcinogenesis process. Orphan nuclear receptors have been shown to be involved in numerous cellular processes, including both at a transcriptional and a non-genomic level. There is a significant emphasis to identify ligands that will interact and modify these nuclear receptors, with the long-term aim of developing novel pharmaceutical therapies. The identification of orphan nuclear receptor ligands will also help increase our current understanding of their role in cellular signaling, by enabling manipulation of these receptors. This review aims to provide a brief overview of some key orphan nuclear receptors which may be involved in colorectal cancer.

Keywords Orphan nuclear receptors · Colorectal cancer · Carcinogenesis · Signaling pathways · Nuclear receptors

Background

Colorectal Cancer and the Hunt for Molecular Targets

Colorectal cancer is the 4th most common cancer in the UK, with 40,695 new cases in 2010 [1].

Overall, there has been a rise in the incidence of colorectal cancer since the 1970's [1]. Despite improvements in treatment and surveillance, almost 50% develop recurrent and/or distant disease. Unknown underlying cellular processes contribute to treatment failure, metastatic disease and ultimate mortality. The identification and understanding of such processes have driven research to discover novel biomarkers and therapeutic targets.

The interplay of chronic inflammation and carcinogenesis is well established. Colorectal cancer belongs to such inflammatory related cancer groups [2–5]. Recent work has highlighted the role of nuclear receptors and co-regulators in the inflammatory to carcinogenesis process; and emphasis on them as therapeutic targets has gained traction [6]. This review will summarize recent progress and understanding of the role of orphan nuclear receptors in colorectal cancer pathogenesis and therapeutic response.

Nuclear Receptors and Disease

Members of the nuclear hormone receptor superfamily have significant regulatory roles in both normal and diseased tissues [3, 7]. Nuclear receptors act as molecular switches, with key roles in diverse cellular processes, including cell proliferation, differentiation and homeostasis [8]. Nuclear receptors are involved in a multitude of pathological processes including chronic inflammation and carcinogenesis, and therefore are an area of intense focus to identify novel therapeutic targets [9–11]. Currently, there are 48 known members of the human nuclear receptor super-family, with approximately half being considered as "orphan" because their endogenous ligands have yet been identified [12, 13]. Previous reports have alluded to the important role that nuclear receptors (oestrogen receptor) have in breast cancer. The identification of the functional roles of oestrogen receptors have resulted in development of selective receptor modulators (tamoxifen/raloxifen),

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which have subsequently been integrated into the treatment of breast carcinoma with significant survival benefit [14]. Therefore the further identification of selective ligands is one of the major goals of orphan nuclear receptor research [15].

Orphan Nuclear Receptors and Adopted Orphan Nuclear Receptors

Twenty-five years ago, the concept of orphan nuclear receptors was first identified, nuclear receptors with no natural ligand [16]. Identification of functional roles for the orphan nuclear receptors may help unravel 'key' molecular processes for human disease, with significant potential health impact [17]. Orphan nuclear receptors have a similar structure to other nuclear receptors, with an N-terminal, an activation function-1 domain (AF1), a DNA binding domain, an activation function-2 domain, a carboxy-terminal and a ligand binding domain. The AF1 domain assists regulatory transcriptional activity of the nuclear receptors [11] and facilitates binding of co-regulatory factors that have a positive or negative effect on transcription processes [3]. The DNA binding domain permits DNA binding which is important in mediating homo & heterodimerization of the nuclear receptor [18, 19]. A hinge region is also an important site for post-translational modification. The carboxy-terminal and the activation function-2 domain are essential for regulating the nuclear receptor transcriptional activity via mediating ligand and co-regulatory binding [3, 7, 11].

Seven structural orphan nuclear receptor families have been described based on their similarities to other nuclear receptors: Miscellaneous, thyroid-hormone receptor like, Retinoid X receptor like, Oestrogen receptor like, Nerve growth factor 1B like and steroidogenic factor like [13]. "Adopted orphans", are receptors which were initially orphan receptors, but for whom ligands have since been discovered. These include peroxisome proliferator-activated receptors (PPARγ), retinoid X receptors (RXR) and liver X receptors (LXR) [11, 20, 21]. These receptors have been shown to have prominent roles in metabolism and inflammation, with functional roles identified in glucose metabolism, homeostasis and atherosclerosis. The identification of adopted orphan nuclear receptors has the potential for the development of receptor modulators to target disease.

Mechanisms of Manipulation of Orphan Receptor Function

There is a considerable focus to identify the orphan nuclear receptors' natural ligands and characterize their physiological and pharmacological benefit. As they lack a natural ligand, the mechanisms to manipulate orphan nuclear receptor function have remained relatively opaque. Key mechanisms for regulation in the absence of a known ligand that binds to the ligand-binding site include alterations in the level of expression of the receptor, phosphorylation and interactions with co-activators or repressors. Molecules that bind to sites other than the ligand-binding site can also regulate nuclear receptor function and are potential targets for therapeutic intervention. For example, 6-mercatopurine (6-MP) has been shown to activate NR4A2(Nurr1) via its AF-1 domain, indicating that NR4A2 may mediate anti-proliferative effects of 6-MP, and therefore NR4A2 is a possible molecular target in the treatment of leukemia [22].

Orphan nuclear receptors: A target for drug discovery

Orphan nuclear receptors are "hidden switches", that underlie many cellular activities but where current knowledge is lacking, including involvement in transcription [15]. There is a significant drive to identify small molecule ligands that will interact and modify these nuclear receptors, in the hope that pharmaceutical interventions will be available for the treatment of a range of chronic disease and neoplasms. The best example of such success was the development of tamoxifen for the oestrogen receptor in treatment of breast cancer [23].

Methods of identification include using cell-based cultured cells transfected with a receptor construct and reporter gene [15]. The transfected cells are then treated with a range of candidate ligands and assayed for activity or response from reporter gene product. Alternatively, ligands can be identified based on direct binding to receptor, which is immobilized on solid support. With cell lysates or compound mixtures being passed over the immobilized target protein, after several washes, the ligand may be revealed using mass spectrometry [15]. Other methods of identification include fluorescence resonance energy transfer assays (FRET), in which successful interactions result in energy transfers and fluorescence changes which are detected by microscopy [24] or using amplified luminescent proximity homogenous assay (Alpha Screen) which will detect luminescent changes on successful interactions [25], and x-ray crystallography studies.

Methods outlined above have successfully identified ligands for retinoid X receptors, pregnane X receptors, PPARs, and steroid xenobiotic receptors [15]. Focus on this area of research has led to the discovery of many more signaling pathways, and its link to chronic disease and neoplasms.

Colorectal cancer and Orphan Nuclear Receptors

Carcinogenesis involves critical changes in cell behaviour including evasion of apoptosis, unlimited proliferation,

angiogenesis, and tissue invasion [26, 27]. Orphan nuclear receptors regulate vital transcriptional activity and therefore may be involved in tumour development [6].

Colorectal cancer is a heterogeneous disease that arises through the aggregate effects of multiple genetic and epigenetic genomic alterations that effect cell growth and differentiation. To date there are at least three major pathways that contribute to colorectal carcinogenesis (chromosomal instability, microsatellite instability and serrated/methylation pathways), but the full mechanism of carcinogenesis still requires extensive investigation as sporadic colorectal cancer still accounts for the majority of cases (~75%) [26, 27]. The current knowledge regarding orphan nuclear receptors in colorectal carcinogenesis and its clinical implications are outlined below.

Examples of Orphan Nuclear Receptors with Potential Roles in Colorectal Cancer

NR4A Orphan Nuclear Receptors

The NR4A subfamily of orphan nuclear receptors is comprised of three members; NR4A1 (Nur77, TR3, NGFI-b), NR4A2 (Nurr1) and NR4A3 (Nor1) [18]. This subfamily has a diverse regulating role in key cellular processes including inflammation, proliferation, differentiation and survival [7, 28, 29]. NR4A receptors act principally as transcription factors, but are also recognized to have non-genomic roles in the cytoplasm, for which their subcellular localization holds important clues [30, 31]. [15, 31]. Furthermore, *Mc Morrow et al*. have identified key roles for NR4A receptors in inflammatory arthritis and psoriasis with both pro and anti-inflammatory effects in a cell and context dependent manner [32].

NR4A2 expression is up-regulated in colorectal cancer when compared with normal mucosa and confers poor prognosis [33, 34]. In vitro studies have shown that NR4A2 inhibits p-53 mediated induction of downstream pro- apoptotic genes like BAX (Bcl-2 family) [34]. Induction of NR4A2 by over-expression of PGE2 is mediated via activation of the cAMP/PKA signaling pathway [36], an important pathway in carcinogenesis [37]. The interplay between cAMP/PKA activation and stabilization of P13/Ras has been shown to inhibit apoptosis in colorectal cancer in addition to blocking cleavage of caspase-3 [38].

NR4A2 further facilitates cancer cell survival and growth by facilitating an adaptive shift in metabolism to fatty acid oxidation when tumours cells are deprived of ATP (36). Fatty acid oxidation and subsequent energy release is via transcriptional integration of the eicosanoid and fatty acid metabolic pathways [36]. Furthermore, vascular endothelial growth factor (VEGF) and cyclin D1 may also be induced by over-expression of PGE2 via Wnt signaling [36]. VEGF is a significant factor in cell proliferation and angiogenesis. NSAIDs use results in blockade of Wnt signaling [4] and down-regulation of osteopontin (OPN) [39]. OPN is also another known indicator of tumor progression and invasion, and confers poor prognosis [4]. Recent studies have shown that the relative risk of colorectal cancer is reduced by ~40% in those taking regular nonsteroidal anti- inflammatory drugs (NSAIDs). This highlights that the blocking cyclo-oxygenase pathways (especially COX-2) may have an important role in preventing colorectal carcinogenesis [33].

A recent study by *Han et al*. observed that NR4A2 expression also confers chemotherapy resistance, especially in patients receiving FOLFOX6. It is postulated that NR4A2 promoted stemness of colorectal cancer cells enabling PGE2 mediated protection from apoptosis, and causing over-expression of epidermal growth factor receptors [34].

NR4A1's induction is caused by deoxycholic acid, a known colorectal carcinogen. There is mounting evidence showing that NR4A1 is a pro-oncogenic factor in multiple neoplasms. NR4A1 protein (cytoplasmic) is over-expressed in breast cancer patients [40], and in colorectal cancer patients [3]. Knockout of NR4A1 has also been shown to causes inhibition of cell growth, with decreased angiogenesis and induction of apoptosis [7]. More recently, NR4A1 has been shown to be linked with beta-1 integrin expression, and a potential therapeutic approach has been shown in vitro with use of C-DIM NR4A1 exogenous ligands [41].

Estrogen Related Receptor Alpha (ERRα)

High expression of the orphan nuclear receptor ERRα has been linked to poor prognosis in colorectal cancer [42]. Liang et al demonstrated that high expression, both at protein and mRNA level was associated with a poor prognosis in colorectal cancer. This is likely a transcripional effect as the increased levels were noted to be nuclear [42]. This is similar to other tumours, e.g. breast cancer where high expression is also associated with poor prognosis.

Retinoid-related Orphan Receptors

Retinoid-related orphan receptors receptors have 3 isoforms, alpha, beta and gamma. Small ligands have been identified using x-ray crystallography studies for these receptors [13].

RoRα (NR1F1)

The tumour microenvironment is critical in colorectal cancer. Xiao et al noted that RORα inhibits colorectal cancer cell proliferation, migration and induction of angiogenesis in response to an adipocyte conditioned media [43]. These links between metabolic, inflammatory and cancer pathways are increasingly important in the context of the current obesity epidemic. ROR α has been shown to be involved in

prostaglandin mediated Wnt signaling, and appears to be a central molecular switch [44, 45].

ROR-β (NR1F2)

Mechanisms have recently been proposed where the Wnt pathway is modulated by targeting ROR-β, in "colorectal cancer initiating cells". This may play a role in tbe early development of colorectal cancer [46].

Retinoic Acid Receptor Alpha (RAR-α)

Epigenetic regulation of RAR-alpha has been linked to adverse pathological features in colorectal cancer [42, 44].

Chicken Ovalbumin Upstream Promoter-Transcription Factor 2 (COUP TF II) (NR2F2)

NR2F2 expression has been associated with a poor prognosis in colorectal cancer, and appears to be linked with SMAD4 signaling [47].

Conclusion

This paper outlines the extensive and critical roles that orphan nuclear receptors play in the development of colorectal cancer. Furthermore, it highlights the potential areas for further research and therapeutic targets that could have significant health impact in numerous chronic diseases, inflammation, and carcinogenesis. With subsequent targeting and identification of orphan nuclear receptor ligands, with will also help increase our current understanding of their role in cellular signaling,

Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest.

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