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Review Article

Current Understanding of the Role of the Quadruplets in Colorectal Cancer Biology – Osteoprotegerin and its Ligands RANK/RANKL/TRAIL

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ABSTRACT

Colorectal carcinoma (CRC) is one of the most prevalent forms of malignant tumors worldwide, with over 600,000 CRC-related deaths occurring annually. Despite major advances in surgical techniques and equipment, chemotherapy and radiation therapy, the prognosis for CRC patients remains poor because of distant metastasis and recurrence. Traditionally, the identification of patients at high risk of recurrence or distant metastasis has depended on pathological characteristics, such as depth of invasion, nodal metastasis, stage group and perforation or invasion of adjacent organs. However, the current tumor-node-metastasis (TNM) classification system is limited in that it cannot offer a prognosis for individual patients. To improve the outcome of patients with CRC, it is crucial to identify cancer-related genes that can serve as predictive and prognostic biomarkers to individualize therapy. Osteoprotegerin (OPG) is a member of the tumor necrosis factor (TNF) receptor superfamily. It has been shown that OPG is involved in the development and progression of human malignancies. This review summarizes our current understanding of the role of OPG in fibrosis and will discuss its potential as a biomarker and/or novel therapeutic target for fibrosis.

Keywords:. Colorectal cancer, OPG, RANK, RANKL, TRAIL

Introduction

Colorectal carcinoma (CRC), which causes over 600,000 deaths each year, is one of the most common types of malignant tumors worldwide.[1] Despite advancements in treatment methods such as surgery, chemotherapy, and radiation therapy, the prognosis for CRC patients is poor due to the likelihood of distant metastasis and recurrence. While the current TNM classification system can identify certain pathological characteristics that may indicate a higher risk of recurrence or distant metastasis, it does not provide a prognosis for individual patients.[2]

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Identifying cancer-related genes that can serve as predictive and prognostic biomarkers for CRC is crucial in order to individualize treatment and improve patient outcomes. Osteoprotegerin (OPG), a member of the tumor necrosis factor (TNF) receptor superfamily, has been shown to be involved in the development and progression of various types of human cancer, including CRC. Three previous studies have examined OPG expression and its role in CRC.[3]

Osteoprotegerin structure and known physiological functions

OPG is the trivial name of tumor necrosis factor receptor superfamily member 11B, based on its main function to counteract bone resorption Structurally, OPG is produced as a 60 kDa-monomer consisting of 401 amino acids. The monomers may also be assembled at the cys-400 residue to form 120 kDa disulfide-linked dimers. Both mono- and dimer proteins harbour a signal peptide, which is cleaved prior to secretion to form active OPG. The structure of OPG also consists of four cysteine-rich pseudo repeats located in the N-terminal and these are responsible for its binding activity to receptor activator of nuclear factor kappa-B ligand (RANKL, Figure 1A) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL, Figure 1B). However, OPG lacks a trans-membrane domain for attachment to cell membranes and is thus only biologically available as a soluble protein,which increases its effectiveness to scavenge available RANKL and TRAIL

OPG is widely recognized for its biological function as a soluble decoy receptor for RANKL (also known as TNFSF11).[5] RANKL will induce

formation of multicellular bodies upon binding to receptor activator of nuclear factor kappa-B (RANK, also known as TNFRSF11A) on the surface of osteoclasts. The multicellular bodies will then degrade the organic extracellular bone matrix, ossein, which primarily consists of collagen and hydroxyapatite.[6] By scavenging RANKL, less degradation of ossein will take place through RANKL/RANK interaction and bone matrix integrity is maintained.[7] The production of OPG and RANKL by osteoblasts is regulated, among others, through transforming growth factor beta (TGF) released from bone matrix upon degradation.[8-10] TGF binds to its receptors on osteoclasts to activate a pathway that induces the secretion of proto-oncogene protein Wnt-1 by the multi-cellular body. Subsequently, Wnt-1 upregulates OPG as well as downregulates RANKL produced by steoblasts via the canonical -catenin-dependent Wnt signalling pathway.[11-13] OPG production is also well-known to be induced by the steroid hormone estradiol,[14] which explains the higher risk of developing osteoporosis in elderly women, in whom the production of estradiol is dramatically decreased. OPG has also emerged as a decoy receptor for TRAIL. TRAIL is an apoptotic factor and known to bind to decoy receptor 1,[15] death receptor 4, and death receptor 5 expressed by awide range of cells. Because of its association with cell death, OPG has been studied in the context of cancer/tumorigenesis and vascularization.[16,17] For instance, OPG was shown to be produced by cancer cells to intercept TRAIL to prevent TRAIL-receptor mediated apoptosis.[18] Inflammation can be an early initiating event of fibrosis and the levels of both OPG and TRAIL were reported to be positively correlated with inflammation. In patients with acute pancreatitis, OPG and TRAIL are elevated. The level of OPG correlated positively with the severity of the disease and the duration of hospitalization; however, this was not the case for TRAIL.[19] These findings suggest that TRAIL is expressed earlier during the inflammatory phase, whereas OPG follows as a feedback and it is more important for the remodelling phase and therefore involved in fibrosis. Patients with chronic kidney disease, the OPG/TRAIL ratio correlated with mortality of long-term disease, with OPG levels increasing over time which was not mirrored by TRAIL. This result also implies that OPG may play an important role in the chronicity of the disease.[20] In addition to RANKL and TRAIL, some information is available on other OPG-binding ligands, which include extracellular matrix constituents like glycosaminoglycans and proteoglycans, and vascular factors von Willebrand factor and its complex with factor VIII. In addition, OPG has been reported to bind to syndecan-1 on myeloma cells, resulting in OPG internalization and degradation. OPG can also stimulate chemotaxis of monocytes by binding to syndecan-1 and stimulating migration towards high concentrations of OPG. Moreover, in endothelial cells, OPG stimulated a dose-dependent increase in the expression of adhesion molecules in the presence of tumor necrosis factor α, which was reflected by enhanced binding of monocytes. Thus, OPG appears to modulate monocyte chemotaxis and migration on multiple levels.[21]

OPG secretion

As a soluble glycoprotein, OPG can be found in most organs in various levels.[22] It is produced by a multitude of cells, including osteoblasts, epithelial cells,[23] vascular endothelial cells,[24] smooth muscle cells,[25,26,27] fibroblasts, and cancer cells.[28] In addition to the regulation of bone integrity, OPG has been shown to play a significant role in arthritis,[29,30] cancer,[31,32] and vascularization. Furthermore, OPG levels can be induced by several factors other than estradiol and TGF, such as vascular endothelial growth factor and stromal cell-derived factor-1 in endothelial colony forming cells, and interleukin-13 in fibroblasts. These findings indicate that OPG is subject to regulation by various factors derived from a vast number of different cell types,which makes local/targeted modification of OPG expression and/or associated effects a difficult task.[33]

Down-regulation of osteoprotegerin expression as a novel biomarker for colorectal carcinoma

Pettersen and colleagues and De Toni and colleagues[34] observed the expression of *OPG* mRNA and proteins in CRC cell lines, Tsukamoto and colleagues[35] reported a significant correlation between OPG expression and aggressive behavior of CRC including depth of invasion and distant metastasis and worse survival rates; they identified OPG overexpression as an independent predictive factor for tumor recurrence. However, these studies did not elucidate the mechanism by which these changes in OPG expression occur. Moreover, Pettersen and colleagues analyzed OPG expression only in CRC cell lines and not in a normal colonic epithelial cell line, raising questions about the meaning of the increase in OPG expression in the CRC cell lines. These issues were reinforced by a recent study by Lu and colleagues, where OPG expression in various cancer cell lines, including CRC and nasopharyngeal carcinoma cell lines, was significantly decreased, or remained undetected. They also showed that promoter hypermethylation of OPG gene promoter was involved in the down-regulation of OPG expression. These results indicated that promoter hypermethylation is a mechanism of OPG down-regulation in CRC, and that OPG is involved in the carcinogenesis and progression of CRC. We suggest that OPG is potentially useful as a prognostic biomarker in CRC. These results suggest that OPG may be a potential tumor suppressor. Restoration of OPG expression may offer a new therapeutic approach for treating patients with CRC.[36]

The role of OPG in inflammation

In addition to bone metabolism, the interactions between OPG, RANKL and RANK also have relevance to inflammatory pathways. RANKL–RANK binding activates several pathways that contribute to the survival of T-lymphocytes and dendritic cells (DC). In addition, OPG is synthesized by DC and B-lymphocytes whereas RANKL is mainly produced by T-lymphocytes. Moreover, RANKL and various cytokines (e.g. Tumor Necrosis Factor (TNF)-α) induce the synthesis of OPG from immune cells. In turn, the interruption of RANK–RANKL ligation by OPG down-regulates T-lymphocyte and DC activity, thereby modulating inflammatory responses.[37]

The role of OPG in TNF-related apoptosis

OPG inhibits the TNF-related apoptosis inducing ligand (TRAIL) pathway, and thereby decreases apoptosis of certain cells. The TRAIL pathway is now well characterized. TRAIL binds certain death receptors (DR4 and DR5) located on the cell surface, 34–36 which then initiates apoptosis. However, three decoy receptors (DcR1, DcR2, and OPG) act to inhibit the binding of TRAIL to either DR4 or DR5, thereby preventing apoptosis. Thus, OPG can act as an inhibitor of apoptosis, particularly in gastric and colonic carcinoma tumor cells.[38] In addition to its role in the induction of apoptosis, TRAIL is affected by the immune system. TRAIL is produced by various immune cells and is regulated by various inflammatory cytokines (e.g. interferon (IFN)-α, and γ).

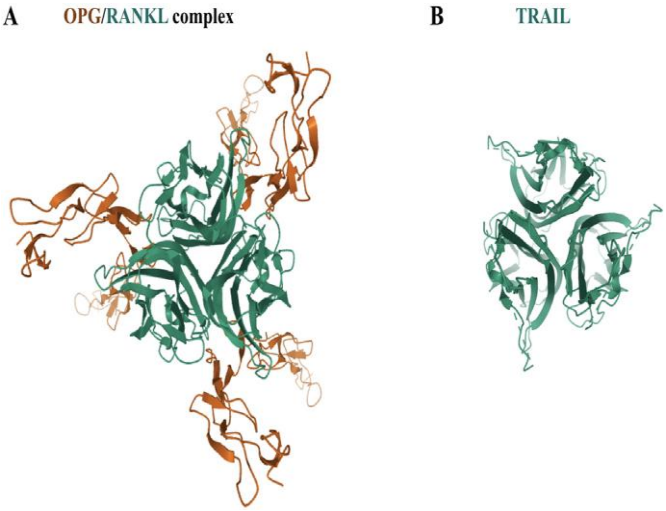

Figure 1: 3D structure of trimeric human RANKL (green) in complex with human OPG (orange). Structures generated on wwPDB (wwpdb.org) (Habibie et al., 2021)

Because of its effect on apoptosis in inflammatory cells, TRAIL was also found to play a role in inflammatory bowel disease, IBD patients by being responsible for apoptosis of enterocytes, thereby disrupting the intestinal epithelium and eliciting an immune response. Furthermore, TRAIL also has a role in bone homeostasis, by inhibiting osteoclast differentiation and inducing apoptosis in osteoclasts. In this context, OPG protects osteoclasts from apoptosis while it paradoxically decreases osteoclastogenesis because of binding to RANKL. However, OPG has similar affinity for both RANKL and TRAIL. In conclusion**,** the complex interactions between OPG and both TRAIL and RANKL provide important regulatory steps in processes related to cell differentiation and cell survival in bone, inflammation and cancer (Figure 2).[39]

Potential contributions of osteoprotegerin to fibrosis development

Although OPG has been found to be upregulated in a multitude of fibrotic organs little is known about its role in fibrosis development.Many effects of OPG are related to its function as a decoy receptor for RANKL and TRAIL; therefore, the impact of these ligands during fibrosis development needs to be considered as well. Several hypotheses regarding the role of OPG in fibrosis development exist, but none have been definitively validated. It has been suggested that RANKL may stimulate the production of extracellular matrix-degrading enzymes, like cathepsins and metalloproteinases, in tissue macrophages in a similar way as it does in osteoclasts. In this way, RANKL could contribute to degradation of extracellular matrix and resolving of fibrosis.[38,39] High levels of OPG may prevent RANKL-induced macrophage assisted degradation of matrix and contribute to the pathogenesis of fibrosis.[40]

Plasma Protein Profiling Reveal Osteoprotegerin as a Marker of Prognostic Impact for Colorectal Cancer

Due to difficulties in predicting recurrences in colorectal cancer stages II and III, reliable prognostic biomarkers could be a breakthrough for individualized treatment and follow-up. Since the detection of carcinoembryonic antigen (CEA) in 1965[41] a large number of biomarker candidates have been proposed to have a potential prognostic impact in colorectal cancer (CRC). However, CEA is still the only serologic marker recommended in surveillance for CRC by experts groups of American society of colon and rectal surgeons [42] and European society for medical oncology.[43] Due to the lack of sensitivity or specificity of the biomarker candidates and due to the polymorphism of the CRC and the tested cohorts none of the suggested biomarker candidates have shown superiority to CEA. The field is extensively expanding due to new analytic techniques such as next generation sequencing, which adds to the complexity of the information. There are several soluble protein biomarker candidates of interest in the prediction of survival and disease recurrences in patients with CRC. However, only one protein, osteoprotegerin, did show a statistical significant association with survival. Osteoprotegerin was the only protein with a significant association with overall survival after correction for age and disease stage. This association was found to be strongest in disease stage III. However, it could not be associated with disease recurrence.[44]

Correlation Between Baseline Osteoprotegerin Serum Levels and Prognosis of Advanced-Stage Colorectal Cancer Patients

Osteoprotegerin (OPG) is a soluble receptor of the pro-apoptotic cytokine TRAIL which is thought to contribute to tumour development by inhibiting apoptosis or affecting other aspects of tumour biology, including cell proliferation and immune response. Although immunohistochemical studies suggest that OPG correlates with survival in metastatic colorectal cancer (mCRC), only scarce data are available on serum OPG in CRC patients. study provides evidence of independent prognostic significance of serum OPG in patients with advanced mCRC and warrants its further prospective validation. According to the proposed role of the TRAIL-system in oncogenesis, OPG is thought to contribute to the development of several tumour entities comprising breast, prostate and gastric cancer.[45] More recently, however, it has been proposed that OPG also affects other mechanisms of tumour formation, including enhancement of cell proliferation and paracrine mechanisms influencing tumour microenvironment.

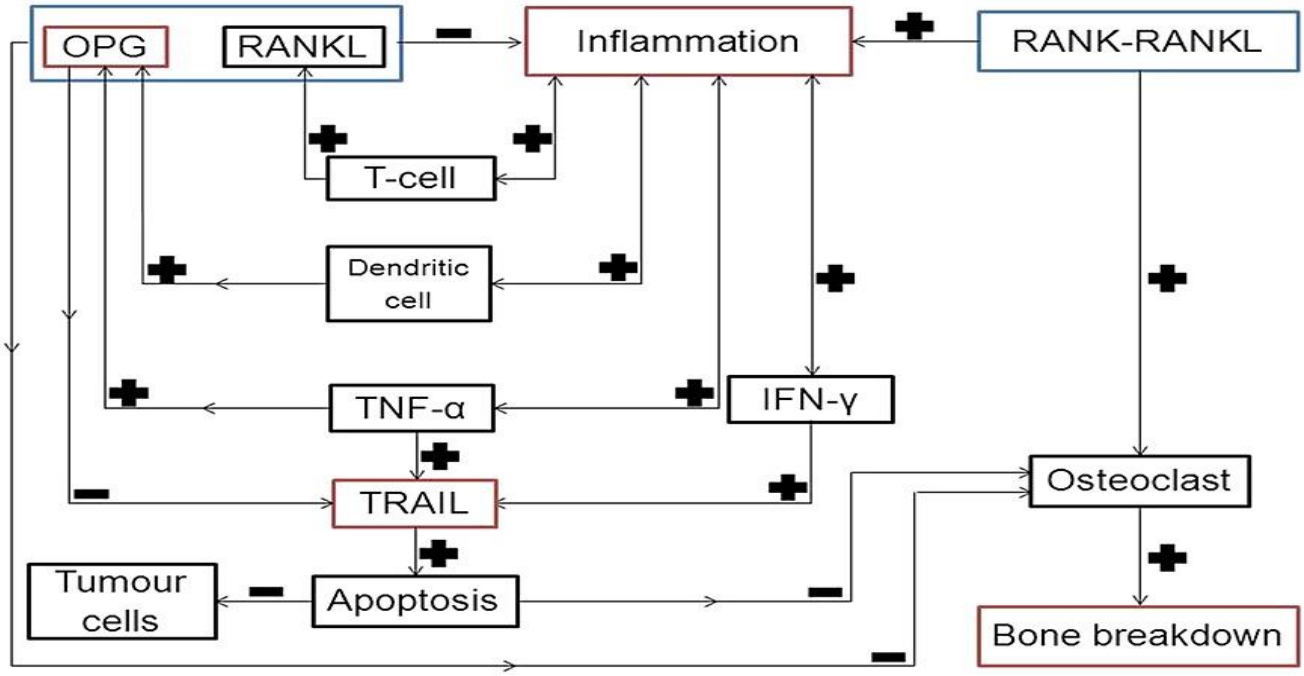

Figure 2: The role of OPG, TRAIL, RANK, and RANKL in inflammation, cell survival, and bone homeostasis. RANKRANKL binding (blue) increases inflammation (red) and bone breakdown (red). In contrast, OPG–RANKL binding (blue) inhibits both inflammation and bone breakdown. In turn, inflammation stimulates T-lymphocytes, dendritic cells, and TNF-α thereby increasing OPG and RANKL concentrations. Furthermore, OPG inhibits TRAIL (red) while cytokines stimulate TRAIL. In this way, OPG increases tumor cell life and osteoclasts.

OPG is a transcriptional target of -catenin in colorectal cancer, and that its concentration is increased in serum of late-stage mCRC patients. Subsequently, basing on mRNA expression analysis of immunohistochemical samples, other authors independently confirmed that OPG is associated with an aggressive phenotype and metastasis formation in colorectal cancer patients. Very recently, by using a protein screening array, Melzer and colleagues [46] independently observed an increase in OPG serum concentration during neo-adjuvant treatment of rectal tumours. These authors reported a trend towards a poorer survival in CRC patients with high baseline-OPG; on the other hand, an increase of OPG during the neoadjuvant treatment was associated to a better progression-free survival. The concept that OPG favours tumour development has been questioned also by recent data showing that lower immunoreactivity for OPG in tissue samples from CRC is associated to a poorer outcome.[47] These data suggest that OPG plays different roles in different stages of tumour development or in different therapeutic settings. Assessment of a cohort of patients with metastatic colorectal cancer shows for the first time that high serum OPG has a prognostic significance in mCRC patients which is independent of the well-established prognostic value of CEA. These data are in agreement with previous immunohistochemical findings provided by Tsukamoto and colleagues, who found that OPG staining was increased in tumours of patients with metastasic disease and was associated with poorer prognosis. The results are also in keep with the Tromsø study, a large Norwegian study which prospectively investigated a large population cohort showing that serum OPG is associated with increased risk of developing cancers of gastrointestinal origin and that OPG predicts cancer-related mortality.[48] This data also confirm the very recent findings by Meltzer et al. showing that high baseline OPG tends to correlate with poor survival in the neoadjuvant treatment setting of rectal cancer. The report is consistent with the proposed role of the TRAIL-system in carcinogenesis,[49] with previous observations from different tumour entities [6-8], with the recent report on pre-therapeutic baseline levels of OPG in rectal carcinoma patients, and with data from a large prospective epidemiological Norwegian study showing that OPG in serum correlates with cancer-related mortality]. The additional recent finding by Meltzer et al. that increasing OPG levels during treatment correlate with a favourable prognosis suggests that OPG may have properties which deserve to be further investigated. In particular, additional studies should assess whether changes in OPG concentration during therapy play a functional role in determining response to treatment or rather reflect increased release of OPG from tumours responding to chemotherapy or radiation-treatment. Confirming a biological significance of OPG in the development of colorectal cancer could open potential therapeutic perspectives: the discovery of a different role of OPG within the OPG–RANKL–RANK system led to the development of denosumab, which is employed to prevent the consequences of bone fragility in patients with bone metastases.[50] In a similar way, antibodies targeting OPG might be used as cancer treatment in tumours overexpressing OPG.

Limitation

The study above has its limitations due to the fact that immunohistochemical and genetic characterization and treatment data could not be retrieved for all individuals of this cohort. However, the pilot study is the first report on the prognostic effect of OPG in pre-therapeutic sera of metastatic colorectal cancer patients and warrant prospective investigation of OPG in serum of patients in different tumour stages and therapeutic settings.[51]

Systemic release of osteoprotegerin during oxaliplatincontaining induction chemotherapy and favorable systemic outcome of sequential radiotherapy in rectal cancer

In colorectal cancer, the influence of the tumor microenvironment with its immune effectors for disease outcome is increasingly acknowledged. The recent study demonstrating favorable survival following immune checkpoint blockade in metastatic disease from mismatch repair-deficient tumors with a high density of immunogenic neo-antigens will obviously be regarded as a landmark contribution to the concept of immune modulation in colorectal cancer.[52]

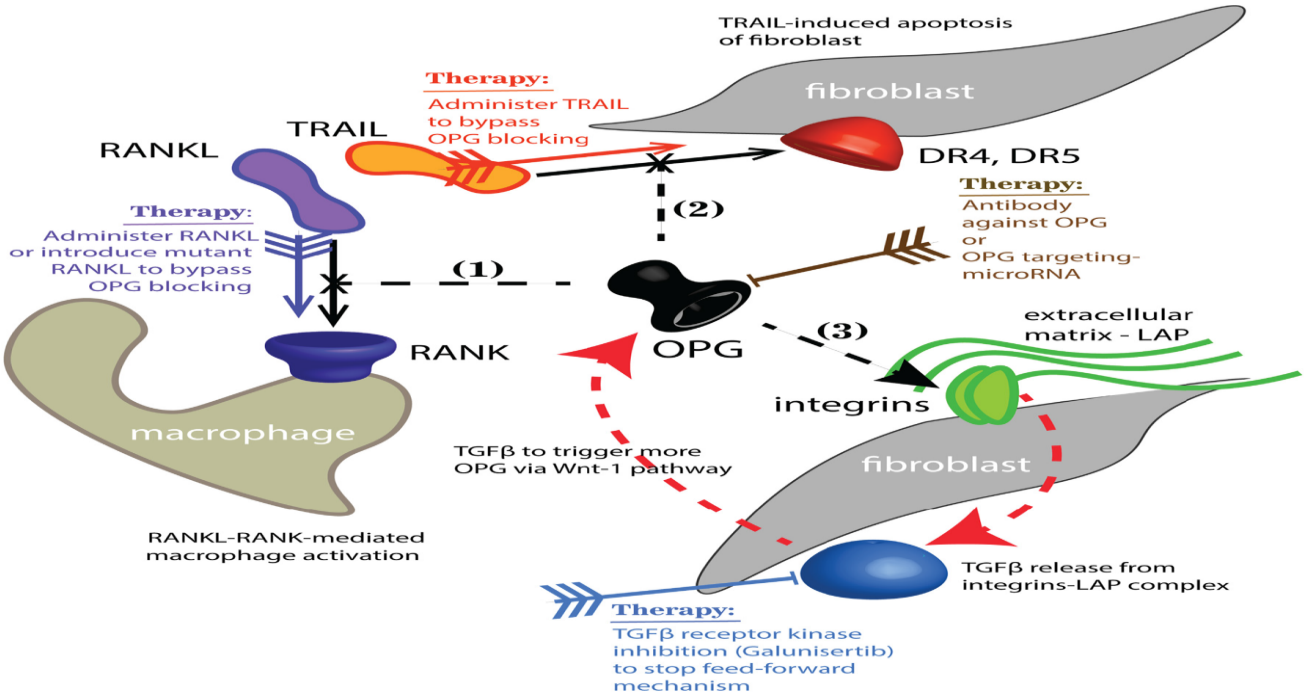

Figure 3: OPG may contribute to profibrotic events in various ways, including, but not limited to, three main mechanisms: (1) as a RANKL decoy receptor to prevent RANKL from activating antifibrotic macrophages by binding to RANK; (2) as a TRAIL decoy receptor to prevent TRAIL-mediated apoptosis of (myo)fibroblasts; and (3) by binding to integrins on the fibroblast surface to release TGF resulting in the TGF-OPG feed-forward loop. Therapeutic approaches targeting OPG signalling to reduce and/or (further) prevent fibrosis can be achieved by reducing free OPG using a therapeutic antibody or OPG-targeting microRNAs, or by the administration of RANKL (or mutant RANKL) and TRAIL creating a surplus of these ligands to bypass the blocking effects of OPG. An indirect strategy could consist of inhibiting TGF receptor kinase (e.g., by galunisertib) to inhibit feed-forward production of OPG by TGF.[54]

Osteoprotegerin as a therapeutic target in organ fibrosis

As described in this review, in addition to serving as a biomarker, OPG itself may contribute to the pathology of fibrosis and may therefore represent a potential therapeutic target. In this context, we will consider and discuss two strategies: (1) neutralizing OPG, and (2) targeting the OPG/RANKL/TRAIL-axis (as depicted in Figure 3). Of note, the OPG/RANKL/TRAIL-axis is involved in a multitude of biological events in a wide range of tissues. Complete knock-out of one of these proteins results in a imbalance of systemic homeostasis with various undesirable manifestations. The use of a therapeutic antibody against OPG has recently been tested as a treatment for pulmonary arterial hypertension in multiple rodent models.[53]

The production of OPG after TGF stimulation. Although this needs to be studied in more depth, it is conceivable that such a negative feedback mechanismis lost or impaired in fibroblasts from patients with fibrosis. Reconstituting these TNFRSF11B-targeting miRNAs would be another way to limit OPG production with possible therapeutic benefit. Examples of indirectly targeting OPG include the use of RANKL mutants with low affinity for OPG that could be used if the RANKL neutralizing activity of OPG is part of the pathological problem; a similar approach could be attempted for TRAIL. In addition, targeting the TGF-OPG feed-forward loop that seems to exist at least for liver fibrosis represents another strategy to indirectly impact OPG functional dynamics.[55]

Conclusion

It was found that OPG can predict, indicate, and initiate many pathways, making it challenging to use as a direct indicator of disease management in IBD and gastrointestinal carcinomas. However, OPG is a key factor in several processes and has the potential to serve as a novel non-invasive biological marker. OPG expression and serum levels are typically elevated in organs, including the colon, rectum, liver, lungs, heart, kidneys, and vasculature, when they become fibrotic. The upregulation of OPG seems to promote fibrotic processes by inhibiting the anti-fibrotic functions of its ligands, which supports the therapeutic potential of targeting OPG. However, direct inhibition of OPG may have severe side effects, such as the development of osteoporosis, so caution should be exercised when pursuing this strategy. Instead, considering OPG as a biomarker rather than a therapeutic target may be more promising. Several studies involving different types of fibrosis in large groups have shown that increased serum levels of OPG are associated with a higher risk of fibrosis and worse clinical outcomes in various diseases. This suggests that OPG may be a useful non-invasive marker for fibrosis in clinical practice.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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