Osteoarthritis

Carpintero-Fernandez, Paula; Varela-Eirin, Marta; Garcia-Yuste, Alejandro; Lopez-Diaz, Inaki; Caeiro, Jose Ramon; Mayan, Maria D.

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Osteoarthritis: Mechanistic Insights, Senescence, and Novel Therapeutic Opportunities

Paula Carpintero-Fernández, PhD,[1,*] Marta Varela-Eirín, PhD,[1,2,*] Alejandro García-Yuste, MS,[1] Iñaki López-Díaz, MS,[1] José Ramón Caeiro, MD, PhD,[3] and María D. Mayán, PhD[1]

Abstract

Osteoarthritis (OA) is the most common joint disease. In the last years, the research community has focused on understanding the molecular mechanisms that led to the pathogenesis of the disease, trying to identify different molecular and clinical phenotypes along with the discovery of new therapeutic opportunities. Different types of cell-to-cell communication mechanisms have been proposed to contribute to OA progression, including mechanisms mediated by connexin43 (Cx43) channels or by small extracellular vesicles. Furthermore, changes in the chondrocyte phenotype such as cellular senescence have been proposed as new contributors of the OA progression, changing the paradigm of the disease. The use of different drugs able to restore chondrocyte phenotype, to reduce cellular senescence and senescence-associated secretory phenotype components, and to modulate ion channel activity or Cx43 appears to be promising therapeutic strategies for the different types of OA. In this review, we aim to summarize the current knowledge in OA phenotypes related with aging and tissue damage and the new therapeutic opportunities currently available.

Keywords: articular cartilage, articular joint, chondrocytes, connexins, Cx43, senescence, therapeutics

Introduction

Osteoarthritis (OA) is the most common form of musculoskeletal disease with significant health care costs and unmet needs on terms of early diagnosis and treatment. Due to the expanding of aging population, together with the increasing levels of sedentary behavior and obesity, the prevalence of chronic OA is expected to continue increasing and duplicate by 2040.[1,2] OA is a multifactorial disease in which biomechanical and biochemical factors are involved in the structural and functional alterations of the whole joint.[3]

The major hallmark of OA is the degradation and finally loss of articular cartilage structure and function.[4–6] However, articular cartilage degradation is accompanied by bone changes, alterations in the synovial membrane, tendons, and all joint tissues, which cause pain, anatomical changes, and swelling, impairing mobility and reducing patient's life quality.[7] Nowadays, OA is considered a disease that affects the whole joint as an organ.[8]

Early diagnosis is critical for effective treatment before facing severe irreversible pathology. In line with this,

[1]CellCOM Research Group, Instituto de Investigación Biomédica de A Coruña (INIBIC), Servizo Galego de Saúde (SERGAS), Universidade da Coruña (UDC), A Coruña, Spain.
[2]European Research Institute for the Biology of Ageing (ERIBA), University Medical Center Groningen (UMCG), University of Groningen (RUG), Groningen, The Netherlands.
[3]Department of Orthopaedic Surgery and Traumatology, Complexo Hospitalario Universitario de Santiago de Compostela (CHUS), Universidade de Santiago de Compostela (USC), Santiago de Compostela, Spain.
*These two authors contributed equally to this work.

different stages of the disease have been stablished comprising from an early OA status where reduced symptoms such as pain is present[9,10] to disability and joint replacement as a symptomatic late- and end-stage OA.[11] Early OA can be defined histologically as having a grade of 1–3 by the OARSI scoring system, which takes into account the depth of degradation into articular cartilage affecting only the superficial and middle zones of the tissue.[12,13] On the other hand, in the later stages of OA, all the joint structures are affected, stretching the joint capsule, causing swelling, more pain, and stiffness, leading to total joint replacement.[11]

Despite the heavy burden of the disease on individuals and health care systems, there is currently no disease-modifying OA-specific treatment authorized yet and, unfortunately, many OA clinical trials fail.[14] Probably, the lack of OA biomarkers, especially biomarkers of early disease, the complexity of the disease, and the different etiologies, limited the development of new and effective therapeutic strategies.

In this article, we will revise the current knowledge on OA phenotypes and endotypes, paying particular attention to the aging and senescence endotype. Furthermore, we will discuss the new therapeutic opportunities available to treat musculoskeletal diseases such as OA based on the use of different disease-modifying drugs able to restore chondrocyte phenotype and/or to modulate the protein channel activity.

OA Phenotypes

OA is a heterogenous and multifaceted disease with several molecular and clinical phenotypes.[15] The identification of different disease subtypes will be helpful to gain a better understanding of the pathways and mechanisms that might be involved in the progression of the disease and to target them more effectively using specific therapeutic strategies.[16] These subtypes can be clinical phenotypes[17] or molecular mechanistic endotypes.[18,19] A clinical phenotype can be defined as a group of patients who have similar clinically observable characteristics for better identifying individuals who are at high risk of progression or who are more likely to respond to a specific intervention. On the other hand, an endotype is a disease subtype defined by distinct pathophysiologic mechanisms, including cellular, molecular, and biomechanical signaling pathways.[17,20,21]

OA phenotype distinctions are confined to those that affect treatment or prevention decisions and to those that clearly have a fundamental effect on the disease biology and etiology.[22] Besides, there are multiple genetic and environmental factors that increase the risk of developing joint alterations characteristic of OA, clinical manifestations of pain, loss of function, and progression to end-stage disease.[23–25] Based on Dell'Isola et al.'s systematic review,[18] there are six different knee OA phenotypes (Fig. 1), which include (a) chronic pain phenotype with central sensitization; (b) inflammatory phenotype; (c) metabolic syndrome phenotype; (d) bone and cartilage metabolism phenotype; (e) mechanical phenotype; and (f) minimal joint disease phenotype. Although they found approximately 84% of the patients could be classified into the six major phenotypes, 12% of the total could only be classified into an inflammatory category based on the MOAKS (Magnetic Resonance Imaging Osteoarthritis Knee Score).

To date, there are a few OA endotypes described, such as aging endotype,[26,27] inflammatory,[28] an endotype associated with metabolic alterations,[29,30] pain endotype[31–33] or an endotype associated with hormonal dysregulations.[34,35] Among the endotypes, recently, a few studies highlighted the importance of the new endotype related to aging or cellular senescence.[26,27] Particularly, this endotype has been described in human samples and in preclinical models[26,27] where not only greater OA severity but also differences in gene expression and pathways represented by these genes have been observed in older mice compared to young mice in an injury-induced OA model,[36] suggesting that the same OA model may result in different phenotypes depending on age as it will be discussed further in this review.

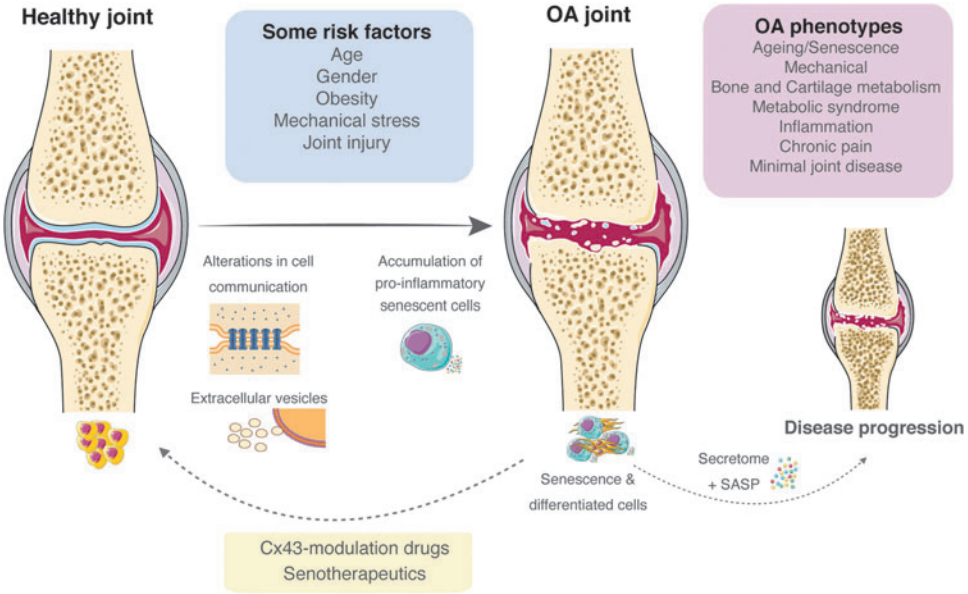

FIG. 1. Several risk factors along with alterations in cell-to-cell communication and the accumulation of senescent cells lead to the development of articular joint diseases such as OA. In the last years, different OA phenotypes were identified, allowing the development of new therapeutic opportunities to treat the disease. The use of senotherapeutics and/or Cx43-modulating drugs can favor a proregenerative environment preventing the progression of the disease. Cx43, connexin43; OA, osteoarthritis.

Furthermore, there are a number of early molecular alterations such as the synthesis of the senescence-associated secretory phenotype (SASP) in chondrocytes from OA patients that causes phenotypic alterations at the cellular level, including chondrosenescence, mitochondria metabolic alterations, and changes in the cartilage secretome involved in the disease progression.[30,36,37] The discovery of an aging phenotype opens the window to develop new therapeutic strategies as it will be discussed later in this review. However, the accumulation of senescent cells in the articular cartilage could be the cause of other OA endotypes as the elimination of senescent cells promotes cartilage regeneration, for example, after injury in young mice.[27] On the other hand, and taking into account the role for cytokines in OA, an inflammatory endotype was defined by Attur et al.,[38] evaluating the gene expression profile of peripheral blood leukocytes.

In the study, the group of patients with higher production of IL-1β showed a worse clinical and structural outcome than patients expressing low levels of IL-1β. In fact, a phase II clinical trial using lutikizumab, an anti-IL-1α/β antibody, in patients with knee OA and synovitis showed limited improvement in pain scores and no change in synovitis in the treated group compared to the control.[39]

In the last years, there was increasing evidence demonstrating a metabolic shift that occurs in the articular cartilage, subchondral bone, and synovium of OA patients, which, in turn, influences the metabolic behavior of chondrocytes, synoviocytes, and bone cells. As a consequence, an endotype associated with metabolic factors was defined.[29,40] Particularly, recent studies have implicated that OA is a metabolic disease associated with several components of the metabolic syndrome like hypertension, type 2 diabetes, and dyslipidemia.[41] During OA, cellular metabolism is compromised and there is an increase in the production of antianabolic, procatabolic, and proinflammatory factors.

The exact metabolic pathways that contribute to structural damage of the joint are not fully elucidated; however, it is believed that elevated adipokine expression from adipose tissue has direct downstream effects leading to cartilage destruction and joint remodeling.[42,43] Also, dyslipidemia has been directly involved in the pathophysiology of OA, aggravating subchondral bone damage.[44] The effect of diabetes can be explained in the sense that diabetic patients experience more synovitis and it has been proven that there is a direct link between the expression of the tumor necrosis factor alpha (TNF-α) in the synovium and insulin resistance in OA patients.[45] Finally, hypertension can contribute to OA development by, for example, depleting blood supply to the subchondral bone culminating in alterations in joint structure.[41]

Although a smaller number of studies explored the pain and hormonal dysregulation endotypes, there is evidence demonstrating that the pain endotype comprises individuals with risk of knee OA that displayed greater feature of sensitization such as pressure pain sensitivity[31,33] and were more likely to develop persistent pain.[46,47] Moreover, it is known that the prevalence of OA increases dramatically in women older than 50 years[48]; for this reason, a few studies reflected the effects of hormone depletion and estrogen treatment in the progression of OA.[34,35] Thus, the data obtained support an effect of endogenous and exogenous estrogen, as well as estrogen receptor polymorphisms on joint health; however, there was insufficient evidence to form stronger conclusions.

The further study of the different OA phenotypes will lead to the development of new disease-modifying OA drugs along with new diagnostic methods that will predict the disease at the molecular and preclinical stages, preventing the progression of the disease.

Main Senescence-Related Molecular Mechanisms in OA

Cellular senescence is a state characterized by a stable cell cycle arrest, associated with macromolecular alterations and usually accompanied by a proinflammatory phenotype or SASP,[49] which involves the release of a heterogeneous mix of cytokines, chemokines, proteinases, and other soluble factors.[50,51] Senescence is a highly heterogenous phenotype,[52] which can be triggered by different stress stimuli, including DNA damage, telomere shortening, mitochondrial dysfunction, or oxidative stress.[49,53] Even there are several generally accepted senescent markers such as cell cycle arrest, high levels of senescence-associated β-galactosidase activity, or overexpression of $p16^{INK4A}$ and $p21^{WAF1/Cip1}$,[54] we still lack universal and specific markers to identify and target senescent subpopulations.

Although the presence of senescent cells is important in a wide range of beneficial process such as tissue remodeling, tumor suppression, and immune surveillance,[55] the accumulation of senescent cells has been also extensively shown to contribute to aging and correlated with the development of age-associated diseases, hence participating in the loss of tissue regenerative capacity with age (Fig. 1).[56,57] The senescent cell phenotype is involved in the progression of OA, as senescent cells have been described to accumulate in cartilage both as a result of aging[58,59] and as a consequence of tissue regeneration after a traumatic injury.[27]

The exact mechanisms by which senescent cells accumulate and participate in OA are still under investigation.[60] Mitochondrial dysfunction and the subsequent accumulation of cellular reactive oxygen species (ROS) result in oxidative stress, which is involved in the anabolic/catabolic imbalance in OA leading to the progressive extracellular matrix (ECM) degeneration.[61] Interestingly, ROS-induced DNA damage is a powerful inducer of NF-kb activation and cell senescence.[62,63] In addition, the SASP includes several proinflammatory cytokines and degradative enzymes (e.g., Il-6, Il-1α/β, Il-8, TNF, MMP-1, or MMP-3), which are also broadly described to induce OA-related changes such as inflammation and ECM degeneration.

Interestingly, the direct association of OA development with senescent cells has been already described by Xu et al.,[64] who transplanted senescent cells into the knee of mice leading to an OA-like phenotype with leg pain, impaired mobility, and histological changes in the tissues from the knee. In addition, the group of J.H. Elisseeff clearly showed in 2017 how the selective clearance of senescent cells in a post-traumatic OA transgenic mouse model halted OA progression, with inhibition of cartilage erosion and proteoglycan loss, together with increased expression of collagen II and aggrecan, as well as reduced observed knee pain.[27]

In this study, the elimination of senescent cells was achieved not only genetically by the specific removal of $p16^{INK4A}$-positive cells but also pharmacologically with the senolytic compound UBX0101, which eliminates senescent

cells, while not affecting the surrounding healthy proliferating cells. In addition to cartilage, senescent cells have been described within other joint tissues such as the bone,[27,31,65] or the synovial tissue.[66,67] Interestingly, connexin43 (Cx43) levels have been demonstrated to correlate with senescent markers in human chondrocytes, including β-galactosidase activity and gene expression of p53/p16^{INK4A} and several SASP factors, such as IL-1β, IL-6, and MMP-3.[68,69] As a result, Cx43 upregulation creates a proinflammatory microenvironment that promotes not only the accumulation of senescent cells through paracrine signaling but also the chondrocyte reprogramming and cellular differentiation toward an immature state, which compromises the ability of these cells to synthesize the ECM components and activates a reversible chondrocyte-mesenchymal transition.[68]

However, this dedifferentiated mesenchymal-like phenotype and the further accumulation of senescent cells can be reverted through the modulation of Cx43 levels. In fact, the genetic manipulation of Cx43 (CRISPR/Cas9 mutant) or the use of a small drug that downregulates Cx43 levels was shown to promote the chondrocyte re-differentiation and resulted in a reduction in the total number of senescent cells, which in the end was correlated with decreased expression of SASP factors and increased expression of cartilage biomarkers such as collagen type II.[68,69] Thus, the use of strategies to downregulate targets, such as Cx43, or the use of senolytic compounds to selectively eliminate senescent cells in musculoskeletal diseases seems to be an effective path to follow as a new and improved treatment strategy,[69–73] together with the use of molecules targeting signaling networks involved in SASP,[68,69,74–76] as it will be discussed later in this review.

Senescence Endotype: Impact of Senescence in OA

Chondrocytes are cells embedded in the articular cartilage responsible for the formation, maintenance, and degradation of components that form the ECM.[77] Based on chondrocyte distribution in the cartilage and due to the ECM structure, it was thought that these cells had no possibility of direct physical contact or communication between each other. However, it has been shown that chondrocytes have long cytoplasmic projections that are able to travel through the ECM and connect two distant cells.[78,79] Besides, articular chondrocytes express several members of a family of channel proteins called connexins (Cxs) implicated in cell-to-cell communication and cell–matrix communication. Particularly, cell-to-cell communication between articular chondrocytes occurs through gap junction channels (GJs) mainly formed by Cx43.[78,79]

Through these channels, chondrocytes are able to exchange small molecules and metabolites such as amino acids, glucose, or small molecules of RNA.[78] It has been reported that chondrocytes isolated from OA patients have higher levels of cell-to-cell communication through these GJ channels when compared with healthy donors.[80] Furthermore, articular chondrocytes are able to form a network of functional GJs with the synovial and bone cells, which can be involved in the metabolic coupling and cellular signaling throughout the whole joint.[81]

On the other hand, other forms of cell communication (Fig. 1) have been proposed to participate in the development of age-related diseases. For example, small extracellular vesicles (EVs)[82,83] and tunneling nanotubes[84,85] can contribute to paracrine interactions and transport of components involved in disease progression such as Cx43 or in the spread of cellular senescence to the neighboring cells.[27] Finally, it has been demonstrated that Cx43 overactivity is implicated in the progression and spread of the disease within the joint by enhancing cellular senescence.[68] Cx43 also forms hemichannels (cell–matrix communication) that participate in the paracrine communication and inflammation by ATP release and other small molecules to the millennium, which might be involved in the progression of the disease as well.[86]

New Potential Therapeutic Opportunities for Senescence Endotype in OA

Although a lot of effort has been put into the development of new therapeutic strategies to prevent and treat OA, the current available treatments are mostly based on nonsteroidal anti-inflammatory drugs or total joint replacement at a critical point.[87] It is widely known that senescence endotype is accompanied by the secretion of different inflammatory factors to the surrounding environment; so the finding of new therapeutic opportunities targeting these two scenarios has been a topic of study during the last years.

More than 50 clinical trials were developed to assess the potential of mesenchymal stem cells (MSCs) to treat OA.[88] For example, intra-articular injections of MSCs directly at the injured site seemed the best strategy to avoid systemic distribution,[89] improving the expression of cartilage regeneration markers such as COL-2a and SOX9, and reducing the expression of destructive markers such as proinflammatory cytokines *in vivo*.[90] However, this treatment showed limited efficacy. To solve this problem, EVs derived from MSCs were explored. The results reported by Zhang et al. and Cao et al. using EVs derived from MSCs increased the therapeutic effect within time in *in vitro*[91] and in *in vivo* models[59]; yet the anti-inflammatory effect was temporal and limited.

Furthermore, EVs derived from MSCs were also reported to be uses in combination with different compounds, such as hydrogels or coating scaffolds.[92] Based on all of these findings, EVs opened a new field to develop novel therapeutic strategies due to their unique physical and biological characteristics, which include high biocompatibility and intrinsic targeting activity. Although EVs could be a promising approach for OA treatment, unpublished results from our group demonstrated that EVs from chondrocytes isolated from cartilage of OA patients promote disease progression through the transmission of inflammatory factors, inducing an OA phenotype in healthy chondrocytes.

There is accumulated preclinical evidence showing the beneficial effect of senescent cell-specific clearance and the use of SASP-modulator drugs for the treatment of musculoskeletal diseases such as OA (Fig. 2). In fact, some of these molecules are being tested in several clinical trials in humans (NCT03513016, NCT04129944, NCT04229225, and NCT04210986). For example, sprifermin (NCT01919164), a recombinant human fibroblast growth factor, reduces the loss of femorotibial cartilage thickness and pain long term.[93] Other drugs, such as those based on the inhibition of

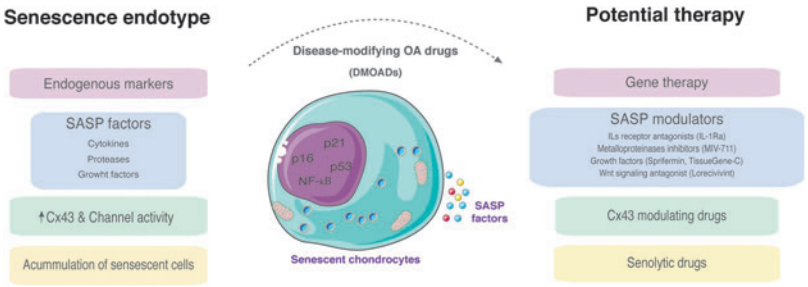

FIG. 2. The senescence endotype is characterized by the accumulation of senescent cell along with the secretion of different SASP factors. Also, increased levels of Cx43 and its channel activity (hemichannels and/or GJs) enhanced this effect (left panel). The use of different drugs able to eliminate senescent cells, to reduce the SASP, or to modulate Cx43 activity (right panel) will be helpful to restore chondrocyte phenotype and to protect the joint from damage. SASP, senescence-associated secretory phenotype.

metalloproteinases (NCT00041756), did not demonstrate the same efficacy, reporting side effects that compromised their safety.[94]

Another approach was based on the neutralization of cathepsin K, which is the major osteolytic protease released by osteoclasts, using inhibitors such as MIV-711, which attenuates joint pathology in a rabbit OA model.[95] Also, when used in OA patients, this inhibitor showed a significant reduction in bone and cartilage OA manifestations, but did not demonstrate any beneficial effect on knee pain in a short-term treatment (EudraCT: 2015-003230-26 and 2016-001096-73).[96] Furthermore, targeting specific SASP factors, including IL-1, IL6, or TNF-α, seemed not to be the best strategy either. A current clinical trial is evaluating the effect and safety of IL1-α receptor antagonist (IL-1Ra) (NCT03968913 and NCT02790723). Other SASP modulators include Wnt signaling antagonists such as lorecivivint,[97] or the use of tissue Gene-C gene therapy[98] improving OA symptoms and cartilage structure.

On the other hand, senolytics are considered a class of new generation therapeutics that selectively eliminate senescent cells. Molecules that are able to reduce Cx43 levels, such as oleuropein, have been reported to improve the phenotype of chondrocytes from OA patients in *in vitro* assays[69] and reduce spontaneous OA lesions in *in vivo* model.[99] Interestingly, oleuropein is also capable of reducing SASP factor levels, including interleukins (IL-1β and IL-6), COX-2, and MMP-3. This effect was also demonstrated in 3D models, where the modulation of Cx43 activity by oleuropein improved the ECM structure, thereby increasing collagen II and proteoglycan deposits.[69]

Moreover, following the demonstration that Cx43 levels are altered in OA and that the protein functioning can depend on its pore conductance, the role of ion channels (voltage-gated and mechanosensitive) in chondrocytes has been explored.[100,101] Interestingly, different ion channel modulators, widely used to treat cardiovascular diseases, for example, showed some beneficial effects in the joint by modulating ionic mechanisms in chondrocytes.[102] Indeed, mechanisms of different pannexins and connexins have been explored, and it has been suggested that inhibition of Ca^{2+}-dependent channels could reduce the progression of the disease.[103] Currently, however, there is no consensus about the possible beneficial effects of ion channel modulators in OA and further investigation is needed.

Finally, it is important to remark that almost all clinical trials described in this review were performed without patient stratification and not taking into account the OA phenotype. This could be the reason why most of them failed giving heterogenous results. To solve this problem, more studies are needed to fully understand the different endotypes and phenotypes to confirm the effect of new therapeutic drugs to prevent and treat musculoskeletal disorders such as OA.

Conclusions

In this review, we have summarized the current knowledge on OA phenotypes, particularly focusing on the senescence endotype along with different therapeutic strategies based on the alterations that suffer the chondrocytes during the OA progression. Although a huge effort has been made from the research community, there is still a need to find different biomarkers and targets to predict the disease at the early stages, to prevent cartilage destruction and develop effective therapeutic strategies. Importantly, Cx43 increase occurs since early stages of the disease[80] and seems to be an effective therapeutic target in preclinical models. Finally, new therapeutic tools adapted to each phenotype and/or endotype need to be developed to protect joint tissues from damage and to increase patient quality of life, reducing social and health burden.

Authors' Contributions

P.C.-F. and M.V.-E. wrote and edited the article with the help of M.D.M. A.G.-Y., I.L.-D., and J.R.C. M.D.M. suggested the topic and revised the article with input from all the authors.

Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Paula Carpintero-Fernández, PhD
CellCOM Research Group
Instituto de Investigación Biomédica de A Coruña (INIBIC)
Servizo Galego de Saúde (SERGAS)
Universidade da Coruña (UDC)
A Coruña 15006
Spain

E-mail: paula.carpintero.fernandez@sergas.es

Marta Varela-Eirín, PhD
CellCOM Research Group
Instituto de Investigación Biomédica de A Coruña (INIBIC)
Servizo Galego de Saúde (SERGAS)
Universidade da Coruña (UDC)
A Coruña 15006
Spain

E-mail: marta.varela.eirin@sergas.es