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Review

Rationale for the use of radiation-activated mesenchymal stem cells in acute respiratory distress syndrome

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Abstract:

Previously we have shown that the combination of radiotherapy with human-umbilical-cord-derived mesenchymal stem-cell therapy significantly reduces the size of the xenotumours in mice, both in the directly irradiated tumour and in the distant non-irradiated tumour or in its metastasis. We have also shown that exosomes secreted from mesenchymal stem-cells pre-irradiated with 2 Gy are quantitatively, functionally and qualitatively different from the exosomes secreted from non-irradiated mesenchymal cells and also that proteins, exosomes and microvesicles secreted by mesenchymal cells suffer a dramatic change when cells are activated or non-activated, with the amount of protein present in the exosomes of the pre-irradiated cells being 1.5-fold times greater compared to those from non-irradiated cells. This finding correlates with a dramatic increase in the anti-tumour activity of the exosomes secreted by pre-irradiated mesenchymal-cells. After the proteomic analysis of the load of the exosomes released from both irradiated and non-irradiated cells, we conclude that annexin A1 is the most important and significant difference between the exosomes released by the cells in either status.

Knowing the role of annexin A1 in the control of hypoxia and inflammation which is characteristic of acute-distress-respiratory syndrome, we have designed a ***hypothetical*** therapeutic strategy, based on the transplantation of mesenchymal stem cells stimulated with radiation, to alleviate the symptoms of patients who, due to pneumonia caused by COVID-19, require the care of an intensive care unit for patients with life-threatening conditions. With this ***hypothesis***, we would seek to improve the patients' respiratory capacity and increase the expectations of their cure.

Keywords: Experimental radiotherapy, radiobiology, Mesenchymal stem cells, Cell therapy, Exosome, Annexin A1, Acute-respiratory-distress-syndrome, COVID-19

1. Introduction

We have recently shown that the combination of human-umbilical-cord-derived mesenchymal stem-cell therapy plus radiotherapy significantly reduces the size of established tumours in mice, both in the directly irradiated tumour and in the distant non-irradiated tumour (1) or in its metastasis (2). These results support the hypothesis that human mesenchymal cells are radiosensitizers for local tumour radiotherapy, and simultaneously, they represent an effective tool for amplifying the systemic effects of radiotherapy. These out-of-target radiotherapy effects, promoted by the human-mesenchymal stem cells (MSCs) are, in our view, of major interest (3-5).

We have also proved (1, 2), that the pre-irradiation of MSCs trigger an important cellular change that transforms the MSCs into a source of molecules with very interesting pharmacologic proprieties. Amongst these actively-secreted molecules, we have identified TRAIL and Dkk3 with very well-known anti-tumour activities, and annexin A1, whose activities we have previously reviewed (3) and, now update here to include new data which demonstrate its anti-inflammatory and anti-viral activities and on its role in the regulation of hypoxia.

This secretion activity suggests a mechanistic explanation of how activated cells may positively spread their effect far from the place where they are applied. On this basis, we believe that exosomes, heavily loaded with annexin A1, will be liberated in the lungs, after cell therapy with irradiated-MSC cells and with this action would ameliorate respiratory symptoms in patients with septic-shock as well as in any other organs affected by septic shock.

A significant number of scientific reports is available demonstrating that gap junction, paracrine pathways and exocrine effects can transmit radiation-induced biological effects far from the place where the radiation is applied. These effects are frequently referred to as radiation-induced out-of-target effects. Multiple molecular signalling mechanisms (6) involving oxidative stress (7, 8), kinases, inflammatory molecules(9, 10), exosomes (2), micro-vesicles are postulated to contribute to bystander short- and long-range effects (3). The anti-cancer immune response may also be activated by ionizing radiation, and a combination of different treatment strategies is promising in this field (11, 12). The activation of the immune system by the irradiated cells to trigger the beneficial abscopal effect are improving, in a decisive way, radiotherapy applications and their outcomes (13-16).

2. Mesenchymal stem cells and radiotherapy

It is generally acknowledged that mesenchymal stem cells (MSCs) can be found ubiquitously in many tissues and are not limited to those of mesodermal origin, such as bone marrow, adipose, muscle and bone (17).

Previous reports suggested a protective role for MSCs when combined with RT (18, 19). In effect, due to their properties, MSCs may be recognized as a therapeutic tool for treating

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radiation-induced tissue damage (20-22). Several reports have shown that MSCs skillfully home onto neoplastic tissues (23, 24) and together with tissue recovery functions MSCs prepare the microenvironment by interactions with molecular inflammatory response to reduce the inflammation grade (25, 26) having the greatest therapeutic impact *in vivo* (27). However, the number of mesenchymal cells that are up-takes into injured tissues may not be sufficient to explain their strong overall protective effect.

The bioactivation of MSCs may be obtained by different ways (28) and the molecules secreted by the activated MSCs (MSCs∗) might have an impact on several of immune cell lineages establishing an advantageous sphere far away of its localization place. We have proposed that exosomes liberating from radiation-activated mesenchymal stem cells perform an important and systemic antitumoral action (1, 2).

On the other hand, it has been attributed to macromolecules included in the exosomes released by the MSC cells the induction of the mechanisms of epithelial and endothelial wound healing and the angiogenesis promotion, as tools for defending the intestines of the damage produced for necrotizing enterocolitis experimentally induced in animal models [29]. This has been highly promising (29-31) and MSCs may be a well-thought-out therapeutic tool to treat radiation-induced tissue damage (32). It is essential to highlight that the group of Chapel et al. has started a phase 2 clinical trial (ClinicalTrials.gov Identifier: NCT02814864) for the handling of severe collateral healthy-tissues damage after radiation therapy in patients with prostate cancer and this clinical trial is sustained by numerous reports focused on the use of MSCs for improving the damage severity on normal tissues after radiation treatments (33-35). However, the damage severity and the mechanisms involved in the control of side-effects after radiotherapy (36) as well as the role of MSC in healthy-tissue radio-protection, are quite unknown.

In that sense, we know that in an uninjured mouse, exogenous intravenously-injected MSCs rapidly accumulate within the lungs and are cleared from this site to other organs, such as the liver, within days (34). While take up in the lungs, the MSCs are able of releasing a wide variety of soluble mediators including anti-inflammatory cytokines (37), antimicrobial and angiogenic macromolecules, and exosomes and microvesicles that are secreted to extravacular space (38). However, the number of MSCs that engraft onto injured tissues may not be enough to account for their robust overall protective effects. All of the above makes us suppose that the amount of MSC cells that engraft onto injured tissues may not be sufficient to account for their robust overall protective effects. In the Figure 1 we have included a graphic summary of the huge widespread actions that mesenchymal cell and mesenchymal activated cells can do.

In figure 1 we have included a graphic summary of the widespread actions that MSC and activated MSC* can do.

3. Radiation-activated mesenchymal stem cells

When we studied the exosome cargo before and after the activation of MSCs with RT, we discovered significant disparities in the results of the proteomic assessment of both samples. We have described that there are qualitative, quantitative and functional differences amongst the proteins contained in the exosomes obtained from basal MSCs and activated MSCs* (39).

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That demonstrates the profound metabolic change that these activated cell exosomes have undergone and the consequences after activation with radiation. Amongst the proteins representatives in MSCs* we highlight the key components of cell-cell or cell-matrix adhesion and include annexin and integrins (39). Between them, the presence of Annexin A1 (ANXA1) (39) is very noteworthy because it is at all times present in the exosomes released from MSCs* and constantly absent in MSCs. We have verified these results using quantitative mRNA-PCR to measure the mRNA of this protein in MSCs and MSCs* and confirmed that mRNA is spectacularly induced in MSCs after irradiation. After measuring quantitatively the mRNAs of the proteins of TRAIL, Dkk3 and ANXA1 in umbilical-cord stromal stem-cells, before and after cell stimulation with 2 Gy low-LET ionizing radiation, our results show a clear increase in their intracellular levels, compared with the levels found in basal situations (see these results in (39), supplementary material, Figure 2) and notice that the levels of mRNA of TRAIL and Dkk3 at 48 are strongly increased in treated cells compared to the basal levels ($P<0.001$), whereas the levels of mRNA of ANXA1 are strongly increased at 24 hours, and dramatically at 48 hours of cell treatment, with the statistical differences found 24 and 48 hour being very significant ($P<0.0001$), which supports the massive presence of ANXA1 in the exosomes released by the radiation-stimulated MSCs.

4. Annexin A1 in the inflammation and hypoxia processes control

We have stated that the existence of ANXA 1 in the exosomes separated from the culture medium of activated MSCs* and the absence of this protein in the medium withdrawn from the non-irradiated MSCs is a relevant outcome in our previous studies (39).

In relation with this protein we would like to highlight that after more than 30 years of research annexins have been strongly established to control immune responses. The prototype component of this family, ANXA1, has been extensively acknowledged as an anti-inflammatory factor involving the cell mobility and cellular responses of various components of the innate immune system (40). However, it is now recognized that ANXA1 has also important implications in maintaining homeostasis, foetal development, ageing process and in the evolution of several diseases such as cancer (41, 42). Inflammation is a tightly regulated mechanism, started following tissue damage or infection. If unrestrained or unsolved, the inflammation may lead to further tissue damage and give rise to persistent inflammatory diseases and auto-immunity with eventual loss of organ function. It is now evident that the outcome of inflammation is an active process that occurs during an intense inflammatory incident (43). After MSC activation, the released ANXA1 diminishes the gathering of neutrophils in the tissue injured by several ways, additionally, ANXA1 promoted neutrophil apoptosis and acts on macrophages to stimulate the phagocytosis and the removal of dead neutrophils (43, 44) and leads to the quick reconstruction of tissue homeostasis. Inflammation resolve is controlled by several endogenous factors, involving macromolecules and proteins, such as ANXA1 and their presence is relevant in many diseases (45, 46). The study of ANXA1 in relationship with the innate immune system has been focused mainly on the anti-inflammatory and pro-resolving actions through its binding to the formyl-peptide receptor 2 (FPR2)/ALX receptor. There is many evidences that

ANXA1, and its mimetic peptides (45), may have a important role in alleviating ischemia-reperfusion injury associated complications (47). Moreover, the presence of chronic inflammation in tumours is common and facilitate tumour growth, metastatic dissemination, and treatment resistance (48). Physical abnormality of tumour vasculature include its chaotic structure, an enlarged interstitial pressure, an increased stiffness and hypoxia which are the physical barriers in tumour treatment (49, 50) and are inspiring new anti-cancer strategies aimed at targeting the tumoral tissue to normalizing these physical irregularities (50, 51).

ANXA1 is an endogenous inhibitor of NF-κB that can be induced in cancer cells and experimental tumours by potent anti-inflammatory glucocorticoids and modified non-steroidal anti-inflammatory drugs (49). In this context, ANXA1 has long been classified as an anti-inflammatory protein due to its actions over leukocyte-mediated immune responses. However, it is now well known that ANXA1 has extensive effects afar the immune system with consequences in maintaining the homeostatic atmosphere within the whole body due to its capacity to influence cellular signalling, hormonal secretion and diseases (49). Upon an injury, epithelial wound shutting is a excellently adjusted process that re-establish homeostasis, but in chronic diseases is related with non-healing vascular lesions; in this processes ANXA1 is implicated as a pre-resolving mediator (50).

Moreover, new studies indicating an intracellular function of ANXA1 are now published. In effect, using AnxA1 knockout mice, is has been noted that ANSA1 is essential for IL-1β release both *in vivo* as *in vitro* (51). Furthermore, we known that ANXA1 co-localize and exactly connect to NLRP3, suggesting the activity of ANXA1 in inflammasome initiation is independent of its anti-inflammatory role via FPR2 (51). These mechanisms, that could be of the major importance in the resolution of lung inflammation and in septic shock through cytokine storm control, deserves more research.

5. Annexin A1 in the treatment of inflammation

Annexin A1 (ANXA1), a 37 kDa monomeric protein, The significance of this protein to the stress response is that its synthesis and release is controlled by glucocorticoids (GCs). After release, has shown that ANXA1 could strongly downregulate polymorphonuclear leukocytes migration into inflammatory places and accelerate their apoptosis, upregulating the monocyte migration into inflammatory sites (52).

Recently the role of ANXA1 in the treatment of acute radiation-induced lung damage has been studied and the causes of its action examined (53). Neuroinflammation initiated by damage-associated molecular patterns, has been implicated in adverse neurological outcomes following lethal hemorrhagic shock and polytrauma (54). Results obtained by Han G et al. (54) offer a proof that attractive pro-resolving pharmacological approaches such as annexin-A1 biomimetic peptides can efficiently attenuate neuro inflammation and reveal a novel complex role for ANXA1 as a therapeutic and a prophylactic drug due to its ability to strengthen endogenous pro-resolving, anti-thrombo-inflammatory mechanisms in cerebral ischemia-reperfusion injury. Finally, it has been announced that recombinant human ANXA1 may represent a novel candidate for the treatment of diabetes type 2 and/or its complications (55, 56).

6. Annexin A1 and lung diseases

Endogenous glucocorticoids are pro-resolving intermediaries, a model of which is the endogenous glucocorticoid-regulated protein annexin A1. Because silicosis is an occupational lung disease typified by persistent inflammation and fibrosis, models regarding this illness have been studied to test the therapeutic properties of the ANXA1 on experimental silicosis (52). The authors have demonstrated that the therapeutic administration of N-terminal peptide of ANXA1 (Ac2-26) in ischemia-reperfusion provoked lung injury might substantially attenuate the lung edema and pro-inflammatory cytokine production reducing oxidative stress, apoptosis, neutrophil infiltration, and lung tissue injury, perhaps via the activation of the N-formyl peptide receptor (52).
A similar result has been published in an experimental study made with animals affected by bleomycin-induced lung fibrosis that were treated with an ANXA1 peptide-mimetic, administrated prophylactically (from day 0 to 21) or therapeutically (from day 14 onward), which improved both signs of inflammation and fibrosis (57). Together these data show a pathophysiological relevance for ANXA1 in lung inflammation and in fibrosis, also and may open up new approach for the pharmacological handling of pneumonia and lung-fibrosis. Currently, the resolution of inflammation once considered, to be a passive process, has recently been revealed to be an active and precisely controlled process. In the resolution stage of acute inflammation, new mediators, counting lipoxins and resolvins, which are members of the specific pro-resolving mediators of inflammation, are release (58).

Acute lung injury and the more severe forms of acute respiratory distress syndrome, ALI/ARDS are relatively common syndromes in seriously ill patients, and are related with high rate of morbidity and mortality. Recently, new evidence has shown that the resolution of inflammation might be an active and highly regulated process. Specific pro-resolving mediators, (SPMs) have been proved to yield strongly immune-resolving effects, such as cell proliferation, migration, clearance of apoptotic cells and micro-organisms. Therefore, the effective and timely control of inflammation could be the key step to maintain effective host defense and restoration of homeostasis. So, this reveals a new mechanism for pulmonary edema fluid reabsorption in which SPMs, amongst them annexin A1, might offer new chances to design "re-absorption-targeted" treatments with high levels of precision in controlling acute lung injury (59). It is also widely acknowledged that edema fluid should be removed for patients with ALI/ARDS to survive (60).

Moreover, lung endotoxemia is characterized by neutrophil accumulation, enlarged vascular permeability and parenchymal damage. In relation with toxic problems it has been proposed that the molecular reactions stimulated by ANXA1 peptidomimetic Ac2-26 lead to the control of leukocyte activation/migration and both cytokine production and lung inflammation are generated by lipopolysaccharides (61). In the same way, it has been published that ANXA1 may accelerate the resolution of inflammation in acute radiation-induced lung damage through the inhibition of IL-6 and myeloperoxidase inflammatory

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cytokines, demonstrating that ANXA1 may have therapeutic potential as a treatment for the radiation of acute-induced-damage (62).

On the other hand, it is well known that pattern recognition receptors (PRRs) are key elements in the innate immune response. FPR2/ALXR, a receptor modulated for specialized pro-resolving mediators of inflammation, amongst them annexin A1, has been shown to be one of the receptors implicated in inflammation process control. That has encouraged the research community to search to search for and to find anti-inflammatory/pro-resolution small molecules to control inflammation by activation of FPR2/ALXR (44).

We believe that, in viral infections, is very important the protective function of the ANXA1-FPR2 signalling axis recently described (62). The formyl peptide receptor (FPR) 2 is a pattern recognition receptor that, in addition to pro-inflammatory, pathogen-derived compounds, also recognizes the anti-inflammatory endogenous ligand annexin A1 (ANXA1) and it has been shown that ANXA1, via FPR2, controls inflammation and bacterial dissemination during pneumococcal pneumonia by promoting host defenses, suggesting ANXA1-based peptides as a novel therapeutic strategy to control pneumococcal pneumonia (63).

In this context, it has been described that mice with the influenza A virus (IAV) infection in the murine model treated with ANXA1 displayed significantly attenuated pathology upon a subsequent IAV infection with significantly attenuated pathology upon a subsequent IAV infection with significantly improved survival, impaired viral replication in the respiratory tract, and less severe lung damage.

7. COVID-19: The magnitude of the problem

Most countries in the world are suffering a significant spread of COVID-19, causing pandemic effects. The clinical presentation of COVID-19 infection varies from asymptomatic or with light symptoms to clinical situations characterized by respiratory insufficiency requiring mechanical ventilation and care in the ICU, to multi-organ disfunction syndrome with signs and symptoms such as sepsis, septic shock, and multisystem failure. And it is true that, unfortunately, all the countries in the world are lacking the capacity to solve this problem due to the lack of therapeutic measures that have the appropriate impact. The problem is massive. Therefore, there is a great need to contemplate new methods to improve patients' biological resistance to COVID-19 by using mesenchymal stem cells (64). We know that COVID-19 invade cells through the ACE2 receptor widely expressed in human cells including the alveolar epithelium and the capillary endothelium. The MSCs are ACE2 negative. So, the transplanted cells cannot participate in the spread of the infection.

For the healthcare services, the two key imperative necessities in the COVID-19 pandemic are to hinder and reduce infection rate, and to decrease the death rate of those infected. The accumulating epidemiological analyses, connected with country-based mitigation strategies, and with estimations that about 80% COVID-19 patients have mild or asymptomatic disease, 14% severe disease, and 6% critically ill, support a permanent need for the treatment of COVID19 pneumonia in the long term.

According to preliminary estimates of severity that were based on a recent analysis of data from EU/EEA countries and the UK available in *the European Surveillance System TESSy*

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and online country reports (for countries whose data was incomplete or missing in *TESSy*) and summarized by the *European Centre for Disease Prevention and Control (ECDC)*, we know that amongst all the cases of patients affected hospitalization has occurred in 32% of cases reported from 26 countries and cases with severe illness (requiring ICU and/or respiratory support) has accounted for 2.4% cases reported from 16 countries. Moreover, amongst hospitalized cases severe illness was reported in 9.2% of hospitalized cases from 19 countries and death occurred in the 11% of the hospitalized cases from 21 countries. The age-specific hospitalization rates amongst all cases showed elevated risk among those aged 60 years and over. Finally, a strong estimate for the COVID-19 case death rate is still lacking and theoretically biased by partial outcome data and differences in testing policies and procedures.

The number of people affected worldwide is progressive and continuously growing, and COVID-10 has infected more than 16,5 million people and killed more than 660.000 people in different countries, areas or territories with cases (ECDC at 29 July). The lethality (average) is ≈ 4,10 % with a range of 0.1 % to 14,0% depending on the country.
The magnitude of the problem is enormous and terrifying.

8. Clinical trials of MSC transplantation in patients with COVID-19 pneumonia

MSC products are quickly arising as promising treatment candidates for COVID-19 in pandemic. It is well known that septic shock is associated with a considerable load in terms of both mortality and morbidity for survivors of this illness. Pre-clinical sepsis studies advise that mesenchymal stem cells (MSCs) may moderate inflammation, improve pathogen clearance and tissue repair, and reduce death. Because MSCs have not been assessed in humans with septic shock, a clinical trial that examines safety and tolerability of MSCs is mandatory before proceeding to a randomized controlled trial to study patient outcomes. That has been performed by L.A. McIntyre et al. (65) and their results show that the infusion of freshly cultured allogenic bone-marrow–derived MSCs, up to a dose of 3 million cells/kg, into patiens with septic shock seems safe and consequently, the results of the phase I dose escalation and safety trial provide researchers with the rationale and argument to now conduct larger trials to study the efficacy of MSCs in a clinical trial in patients with septic shock (66), the clinical trial is registered with the www.clinicaltrials.gov (NCT02421484) reference.
Pre-clinical and preliminary clinical data suggests that UCS-MCS because of their anti-inflammatory and immunomodulatory actions are able to heal tissues affected thus improve recovery rate (67). Additionally, this treatment also seems to be anti-microbial. Two recent studies from China (64, 68) have examined if mesenchymal stem cells (MSC) could be useful for treating COVID-19 pneumonia, based on known immune modulatory and reparative abilities of stem cells. Both studies show a outstanding reversal of symptoms, even in severe to critical circumstances. These clinical studies not only recognize a novel therapeutic approach, but also the reality of natural processes able to reduce acute inflammatory pneumonia.

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Following the intravenous transplantation of MSCs, a noteworthy population of cells accumulates in the lung, which together with their immunomodulatory effect, could protect alveolar epithelial cells, recover the pulmonary microenvironment, avoid pulmonary fibrosis, and cure lung dysfunction. It has been suggested that MSCs have cured or significantly improved the functional outcomes of seven patients without any detected side-effects. The pulmonary function and symptoms of these seven patients were significantly improved in 2 days after MSC transplantation. Furthermore, the gene expression profile revealed MSCs were $ACE2^-$ and $TMPRSS2^-$ which shown MSCs are free from the COVID-19 infection. Thus, the intravenous cellular transplantation was safe and efficient for handling in patients with COVID-19 pneumonia, particularly for the patients in a seriously severe condition (64). Given the uncertainties in this area, Golchin et al. (69) have reviewed published clinical trials and hypotheses to offer useful information to researchers and those involved in stem cell therapy. In their study, they considered a new approach to enhance patients' immunological responses to COVID-19 using MSCs and debating the aspects of this proposed treatment. However, at this time, there are no approved MSC-based approaches for the prevention and/or treatment of COVID-19 patients nevertheless clinical trials are ongoing.

The immunomodulatory and anti-inflammatory properties of MSCs in the treatment of respiratory diseases have been confirmed by 17 completed clinical studies, and also more than 70 trials are registered in this regard (https://clinicaltrials.gov).

In this clinical study it is key to only handle with well characterized and safe MSCs including in the most urgent and experimental treatments. Moreover, in order to alleviate in patients with COVID-19 infection the obvious risk of adverse thrombotic reactions after transplant of high doses of poorly typified cell product, has been proposed as obligatory a set of significant procedures for combining innate immune hemocompatibility examination into the usual patients characterization and clinical procedures, before to applied MSC cell therapies (70).

Many of the critically ill COVID-19 patients are in a hypercoagulable procoagulant situation and at high probability for disseminated intravascular coagulation, thromboembolism, and thrombotic multi-organ catastrophe, another cause of high death rate. Therefore, it is mandatory to only use well-characterized, safe MSCs including in the most urgent and experimental treatments (71).

Cost-effectiveness and the speed of medicinal formulation and transport, are themes to be considerate for the MSC based therapy for COVID-19, but whatever the price without a doubt the life of a human is inestimable. Nevertheless, the clinical use of MSC therapy to treat COVID-19 seems promising. Therefore, having in mind that MSC therapy could become an important contribution to reach the end to the high COVID-19 death rates and preventing long-term functional side-effect in those who survive disease, has been demanded that the funding agencies to invert more into development MSCs suitable for the clinical and sure applications (71).

However, it is very important to underline that scientists are tirelessly trying to obtain a vaccine for COVID-19, as well as therapeutics to treat this disease (69) and that now a vaccine to protect against COVID-19 has been assessed for safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 (Ad5) vectored COVID-19 vaccine expressing the spike glycoprotein of a grave acute respiratory syndrome coronavirus 2

(SARS-CoV-2) variety (DOI:https://doi.org/10.1016/S0140-6736(20)31208-3). These recently publish results, shown the vaccine is safe and immunogenic at 28 days post-vaccination. Humoral responses against SARS-CoV-2 hit the highest point at day 28 post-vaccination in healthy adults, and quick specific T-cell responses were observed from day 14 post-vaccination. These findings imply that the Ad5 vectored COVID-19 vaccine deserves more research (DOI:https://doi.org/10.1016/S0140-6736(20)31208-3) and an ongoing phase 2 trial in China (NCT04341389) will offer more data on the safety and immunogenicity of the Ad5 vectored COVID-19 vaccine. The progress in that field is being extremely fast, and an excellent bringing up to date on that subject can be found in (72).

9. Conclusions and perspectives

The present global health crisis involving the appearance and rapid spread of a new coronavirus has encouraged the world scientific community to consider how it can help to combat this mounting viral pandemic.

Amongst all the different mesenchymal cells that might be used, umbilical-cord stem cells seem to be the most desirable for a series of reasons that have been very well explained in (67). Considering together both the previous reports and our own knowledge and research on the exceptional abilities of proliferation (1, 73), secretion (74), and differentiation (17, 71) of the umbilical-cord mesenchymal cells that we have investigated (1, 39), we have also decided to recommend umbilical cord mesenchymal stem cells as a vehicle for annexin A1 for septic shock treatment.

The activation of these types of cells with a 2 Gy low-LET radiation dose, produce an important increase in the cell-released exosomes and these nano-vesicles, which can reach all the tissues and organs affected, contain a very specific load of proteins, including annexin A1(2, 3), whose activity in situations of infection, inflammation and hypoxia, has been intensively discussed in previous pages. This protein together with the endothelium-repair functions characteristic of MSCs, must play a major role in the treatment of the septic shock and pneumonia related with the COVID-19 infection.

Moreover, it is generally accepted that the efficacy of transplanted MSCs actually seems to be independent of the physical proximity of the transplanted cells to damaged tissue. Supposed to be a vectorized signalling system, we believe that the exosomes released from radiation-activated-MSCs cells can reach other organs different from the lungs where they will up-take after intravenous injection and so, extend the anti-inflammatory and anti-microbiological effects of the treatment, to cover systemic problems such as the treatment of patients with septic shock in general and for COVID-19, at this particular time.

This hypothesis provides a rationale for the therapeutic efficacy of MSCs and their secreted exosomes in patients with clinical conditions characterized by respiratory failure necessitating mechanical ventilation and medical assistance in the intensive care unit, for multi-organ insufficiency and systemic manifestations such as sepsis, septic shock, and multiple organ dysfunction cases.

Finally, a scheme for our hypothetical cellular therapy in patients with ARDS would be an intravenous infusion of 6 million/Kg of patient-weigh divided into two parts: a) 3 million non-irradiated-MSC/Kg of patient-weigh, to take advantage of the protective, regenerative

and repair MSC-effects at the lung-vasculature and b) 3 million pre-irradiated-MSC*/kg of patient-weigh, to achieve as soon as possible within the patients the loaded-exosomes with ANXA1 that clinical grade umbilical cord MSC* are able to produce after radiation-stimulation and thus, use the extensive range of anti-thrombo-inflammatory, anti-viral and immunomodulatory actions associated with this protein.

This stem-cell therapy, whether alone or in combination with other therapeutic tools, may possibly be one of the most ideal approaches for treating COVID-19 patients with acute respiratory distress syndrome and
septic shock.

10. Patents

In an attempt to take our basic and regulatory research to clinical practice, we proceeded to apply for the registration of the patent P201500022 and title “Activated stem cells and medical uses,” with the priority date of December 2014. Its international extension via PCT has the number PCT/ES2015/070951 (WO/2016/102735) and was published in June 2016.

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Figure 1

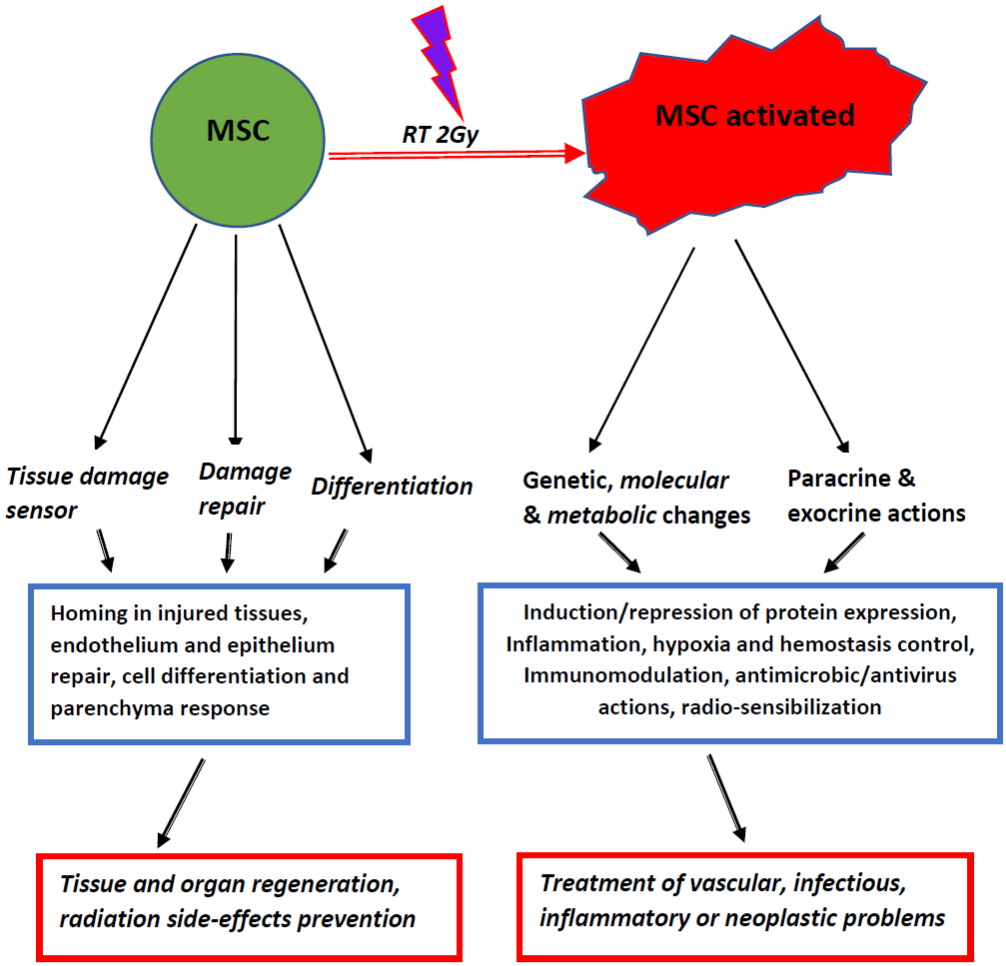

Figure 1: Graphic and schematic summary of cell actions, tissue response and possible therapeutic application of MSC and activated MSC.

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