Campagne 2020 Contrats Doctoraux Instituts/Initiatives

Proposition de Projet de Recherche Doctoral (PRD)

Appel à projet ISVI - Intitiative Sces du vivant ses interfaces 2020

Intitulé du Projet de Recherche Doctoral :
Towards optimized bifunctional senolytic compounds: design, evaluation, and proof of concept during skeletal muscle stem cells ageing

Directeur de Thèse porteur du projet (titulaire d'une HDR) :

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Unité de Recherche :
Intitulé : Adaptation Biologique et Vieillissement
Code *(ex. UMR xxxx)* : 8256

ED515-Complexité du Vivant
Ecole Doctorale de rattachement de l'équipe & d'inscription du doctorant :

Doctorants actuellement encadrés par le directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) : 2 thèses en cours : 1 avec première inscription octobre 2017 soutenance prévue octobre-novembre 2020, 1 thèse programme IPV (50%), première inscription octobre 2018

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Unité de Recherche :
Intitulé : Laboratoire des Biomolecules
Code *(ex. UMR xxxx)* : 7213

ED406-Chimie Moléculaire Paris Centre
Ecole Doctorale de rattachement : Ou si ED non Alliance SU :

Doctorants actuellement encadrés par le co-directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) : 1 thèse programme IPV (50%), première inscription octobre 2018

Cotutelle internationale : ☒ Non ☐ Oui, précisez Pays et Université :

Description du projet de recherche doctoral *(en français ou en anglais)*

3 pages maximum – interligne simple – Ce texte sera diffusé en ligne

Détailler le contexte, l'objectif scientifique, la justification de l'approche scientifique ainsi que l'adéquation à l'initiative/l'Institut.

Project context:

Cellular senescence can be defined as a state of terminal growth arrest triggered by stress signals such as DNA damage, telomere shortening, oncogenic mutations, mitochondrial dysfunction, or inflammation. In addition to this irreversible arrest of cell proliferation, senescent cells (SCs) exhibit an enlarged morphology, distinct metabolic and gene expression profiles and secrete a broad spectrum of proinflammatory cytokines, angiogenic factors, and extracellular matrix proteases, a feature collectively named senescence-associated secretory phenotype (SASP)[1]. While in young organisms such factors are critical for tissue remodeling and suppression of fibrosis and tumorigenesis, the chronic and excessive accumulation of SCs in tissues of older organisms was found to trigger or exacerbate ageing-associated declines and diseases[2]. Indeed, use of a genetic mouse model in which senescent cells were selectively depleted has revealed enhanced longevity and healthy lifespan, together with a reduction in tumorigenesis[3]. With these evidences, a major goal in the field is to design molecules, called senolytics, which can specifically and efficiently eliminate senescent cells. Several senolytics have been identified over the last years and represent promising therapeutical approach, one of them being recently entered into clinical stage[4]. Despite these results, a major obstacle to their use in clinic is their lack of specificity: among the small number of molecules currently available, a large majority does not specifically target senescent cells, leading to non-specific effects. In addition, mechanisms of action by which senolytics function are still not understood rather general pathways related to oxidative stress are proposed. Dissecting out these mechanisms and identifying a way to specifically target senescent cells is thus mandatory to design effective and selective senolytics. Piperlongumine A is a natural alkaloid isolated from vegetal species of *Piper*, which has been reported to have cytotoxic, genotoxic, anti-angiogenic, anti-tumor and more recently senolytic activities[5-7]. While precise mechanism leading to piperlongumine cytotoxicity is still not understood, two studies have reported inhibition of the ubiquitin-proteasome system[8,9]. In addition, we recently provided a direct evidence for its role and that of two analogs in immunoproteasome inhibition[10]. Given the central role of this form of proteasome, induced by oxidative stress and inflammation in senescent cells[11], we now make the hypothesis that the senolytic function of piperlongumine could act through inhibition of immunoproteasome.

In a search for markers of senescent cells, recent studies, using fibroblasts[12] and endothelial[13] cells as models of replicative and oxidative senescence, have shown that expression of dipeptidyl peptidase 4 (DPP4) is strikingly upregulated when cells enter senescence. In addition, the specific overexpression of this serine exopeptidase localized at cell surface, lead senescent cells being highly sensitized to cytotoxicity by natural killer cells in contrast to normal cells[12]. Dpp4 may thus constitute a useful biomarker of senescence, opening new perspectives for a selective targeting.

Among the tissues which function is affected by senescence, skeletal muscle has emerged as an experimental model to study the decline in function of old tissues and to explore rejuvenation strategies. One characteristic of this tissue is its remarkable regenerative capacity after injury thanks to a population of quiescent stem cells, called satellite cells. Upon injury, satellite cells, marked by Pax7 expression, exit quiescence and proliferate, giving rise to a population of committed progenitors capable of differentiation into new myofibers to replace damaged tissue[14]. This regenerative capacity is greatly affected during ageing, associated with a decline in satellite cell functionality[15]: a fraction of the satellite cell population become senescent, a state that leads to a reduced capacity for activation and expansion after injury and to the production of an insufficient progeny to sustain muscle regeneration[15, 16]. Among the therapeutical strategies, selective elimination of senescent satellite cells represents a promising approach[17], allowing the pool of non-senescent satellite cell to efficiently activate, proliferate and expand into committed progenitors.

Objectives of the project:

During the course of this phD project**,** three main objectives will be addressed: (1) the design of bifunctional compounds that could selectively target senescent cells while avoiding non-specific effects using DPP4 as a biomarker (2) the evaluation of their senolytic effect and their mechanism of action *ex vivo* on geriatric muscle stem cells and (3) the establishment of the initial proofs of concept of bifunctional compounds *in vivo* during skeletal muscle regeneration in aged mice.

This interdisciplinary project at the interface of medicinal chemistry and biology with a high potential in translational research is ideally included in the ISVI call for projects. We propose

indeed an elaborated molecular and integrated approach gathering two research teams with complementary skills: team "Integrated cellular aging and Inflammation" (IBPS, B2A) and team "Peptides, glycoconjugates and metals in biology" (LBM).

1. **Design, synthesis and *in vitro* evaluation of bifunctional senolytics** (Years 1 and 2, R. Moumné, C. El Amri) The bifunctional compounds will result from the bridging of two molecular entities, namely piperlongumine (PL) with a selection of well-known clinical inhibitors active-site of DPP4 through an enzymatically cleavable linker (ester or amide group, Fig.1).

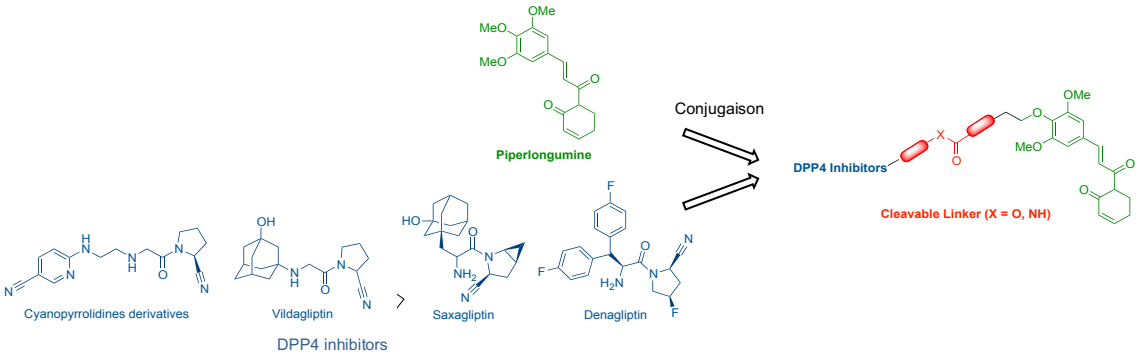

Figure 1: Senolytics and DPP4 inhibitors for the design of bifunctional senolytics.

As the cleavage by cellular esterases or proteases is required to ensure the intra-cellular delivery of senolytic entities[18], various linker lengths and chemical natures will be designed. To select hits for future *ex vivo* and *in vivo* analysis, the different resulting compounds will then be evaluated *in vitro* for both their affinity and their inhibitory effect for recombinant DPP4. Biacore analyser will be used to monitor real-time interactions between DPP4 and the newly synthetized compounds and to determine their affinity constant (K_D), while DPP4 inhibition by these compounds will be quantified by activity assays, using commercial fluorogenic substrates. This chemistry part of the project will be conducted under the supervision of Dr R. Moumné in the Laboratoire des Biomolécules, and of Pr C. El Amri in the Laboratoire de Vieillissement Cellulaire Intégré et Inflammation, who are respectively experts on medicinal and peptide chemistry[19] and protease's enzymology[10].

2. ***Ex vivo* evaluation of the senolytic effect of piperlongumine and bifunctional compounds** (Years 1 and 2, A. L'honoré, C. El Amri).

a. *Senolytic effect of bifunctional compounds compared with primary senolytics*

The senolytic effects and the specificity of selected bifunctional compounds will then be evaluated *ex vivo* on muscle stem cells and compared to piperlongumine alone. Satellite cells will be purified by flow cytometry from adult (6 months-old) and geriatric *Pax7-nGFP* mice[20] (24 months-old) and cultured for 96h to induce their differentiation (adult) or their senescence (geriatric)[14-16]. Piperlongumine, bifunctional compounds or vehicle will be added to culture media using a range of concentration in order to establish a dose-response and to determine an optimal concentration. The senolytic potential of the different compounds together with their specificity will be evaluated at different time points by quantification of apoptotic cells using immunofluorescence and western-blot (Tunel assay, activated-caspase 3 antibody).

b. *Deciphering the implication of proteasomes in piperlongumine and bifunctional compounds senolytic effect:*

To investigate whether proteasomes (constitutive and immunoproteasome) inhibition is involved in piperlongumine and bifunctional compounds senolytic process, both their expression and activities will be evaluated in satellite cells using a range of concentration around the optimal dose obtained above. The accumulation of ubiquitinated proteins and the expression level of proteasomal sub-units will be evaluated by western-blot. The intracellular activity of proteasome will be quantified using luminescent assays (Proteasome-Glo^{TM})[10]. Since piperlongumine is known to have a pro-oxidant activity[5], cytoplasmic and mitochondrial ROS levels will be measured by cytometry using CellRox and Mitosox probes[16].

This first biological part of the project will be conducted in the Laboratoire de Vieillissement Cellulaire Intégré et Inflammation, under the supervision of Dr A. L'honoré and Pr C. El Amri who are respectively expert on muscle stem cells[15] and proteasome[10].

3. **Proof of concept of the senolytic effect of piperlongumine and bifunctional compounds on mice model during ageing** (Years 2 and 3, A. L'honoré)

The senolytic potential of piperlongumine and bifunctional compounds will then be evaluated *in vivo*. Adult and geriatric *Pax7-nGFP* mice mice will be treated with piperlongumine, bifunctional compounds or vehicle by oral gavage for 3 consecutive days every two weeks[21]. Three weeks after the first administration, diaphragm and *Tibialis Anterior* (*TA*) muscles will be used for immunofluorescence on cryosections to count senescent (SA-βgal, p16, pp38 and Pax7-positive cells) and non senescent (Pax7-positive cells) satellite cells. Other skeletal muscles will be used for purification of satellite cells by flow cytometry and analysis of their proliferation and differentiation potential[15,16,20] in culture. Finally, we will investigate the functional consequences of senescent satellite cells depletion using regeneration experiment. After oral gavages similar to those described above, adult and geriatric mice will be subjected to *TA* muscle injury using cardiotoxin intramuscular injection[16]. The regenerative capacity of *TA* muscle will then be evaluated by histology 10 days and three weeks after injury[16]. This second biological part of the project will be conducted in the Laboratoire de Vieillissement Cellulaire Intégré et Inflammation, under the supervision of Dr A. L'honoré who is expert on skeletal muscle regeneration[16].

We expect this PhD project to bring new data on (1) the mechanism by which piperlongumine exert its senolytic effect, with a particular emphasize on the role of proteasome inhibition in this process, and (2) to identify potent senotherapeutics with improved selectivity toward senescent cells using the biomarker DPP4. Using *in vitro*, *ex vivo* and *in vivo* approaches, this project will provide new insights for the design and the optimization of second generation of senolytics with a controlled mechanism of action specifically targeted to senescent cells for application in regenerative medicine. If successful, we could envisage to extent this strategy to other ageing-associated diseases.

Student profile:

The PhD fellow will be involved both in the design/synthesis and the evaluation of senolytics candidates on the biological models of increasing complexity to bring the proof of concept. The research will be conducted simultaneously in both teams at B2A and the Biomolecule's Laboratory. We are looking for a strongly motivated student holding ideally a master M2 in biochemistry and cell biology with a great interest for researches at the interface between chemistry and biology.

References

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