O2.2: Exosome-mediated *MEK1* silencing is a promising approach against triple negative breast cancer regression

D. Ferreira[1]*, C. Santos-Pereira[1;2], M. Costa [3;4], J. Afonso[3;4], A. Longatto-Filho[3;4;5;6], F. Baltazar[3;4], R. Kalluri[7;8;9], J. N. Moreira[10;11] and L. R. Rodrigues[1]

[1]*CEB - Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal*
[2]*Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, 4710-057 Braga, Portugal*
[3]*Life and Health Sciences Research Institute (ICVS), University of Minho, Campus of Gualtar, Braga, Portugal*
[4]*ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal*
[5]*Molecular Oncology Research Center, Barretos Cancer Hospital, Barretos, São Paulo, Brazil*
[6]*Laboratory of Medical Investigation (LIM 14), Faculty of Medicine, São Paulo State University, S. Paulo, Brazil*
[7]*Department of Cancer Biology, Metastasis Research Center, University of Texas MD Anderson Cancer Center, Houston, Texas 77005, USA*
[8]*Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA*
[9]*School of Bioengineering, Rice University, Houston, TX, USA.*
[10]*CNC – Center for Neurosciences and Cell Biology, Center for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, Faculty of Medicine (Polo 1), Rua Larga, 3004-504 Coimbra, Portugal*
[11]*FFUC – Faculty of Pharmacy, CIBB, University of Coimbra, Pólo das Ciências da Saúde, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal*

*e-mail: deboraferreira@ceb.uminho.pt

Introduction

Breast cancer is a major public health problem worldwide being the most diagnosed cancer in women [1]. Triple negative breast cancer (TNBC) represents 10-20% of all breast cancers and is characterized by the absence of hormone receptors (progesterone and estrogen) and lack of expression of epidermal growth factor receptor-2. Furthermore, it is also described to have poor prognosis due to its propensity to metastasize to visceral organs early in the clinical course [2–4].
In accordance with the lack of recurrently altered targets at the genomic level, there is a shortage of approved targeted agents for TNBC, remaining cytotoxic chemotherapy the mainstay of treatment. Even though, various distinct molecular signaling pathways have been implicated in TNBC phenotypes. Of these, aberrant activity of the mitogen-activated protein kinase/ extracellular signal-regulated kinase (MAPK/ERK) signaling activity is revealed to be important in the initiation and progression of cancer [5,6]. Therefore, we sought to uncover the potential of MAPK/ERK cascade downregulation in TNBC through the use of an exosome-mediated system loaded with RNA interference (RNAi)-based therapeutics.

Materials and Methods

Exosomes derived from BJ cells were isolated by differential centrifugation and further characterized by nanoparticle tracking analysis, transmission electron microscopy, flow cytometry and western blot. Moreover, *in vitro* uptake experiments and biodistribuition experiments were accomplished to assess performance. These exosomes were further electroporated with MEK1-targeting siRNA (iexo^{MEK1}), being the impact of downregulation in the metastatic phenotype of highly invasive breast cancer cells evaluated by measuring migration, invasion and proliferation. Changes in the regulation of direct and indirect genes were also assessed. Moreover, implantation of TNBC cells treated with iexo^{MEK1} in chicken embryo chorioallantoic membrane (CAM) was also executed.

Results and Discussion

We showed that MEK1-targeting siRNA (siMEK1) led to an efficient downregulation of *MEK1* in different TNBC cell lines, but the effects on the downstream MAPK/ERK cascade are different. Moreover, we also demonstrated that the siMEK1-mediated silencing led to a cell proliferation impairment that can be supported by the induction of apoptosis for all TNBC cell lines under study, including MDA-MB-231, MDA-MB-157 and Hs 578T.
In our study, we also showed a clear decreased ability of all TNBC cells to migrate and invade upon *MEK1* downregulation. Such impairment in migration/invasion was in part explained by the reversion of the epithelial-mesenchymal transition (EMT) phenotype, characterized by the loss of the mesenchymal markers, including vimentin and n-cadherin and by the gain of epithelial markers, namely e-cadherin. Additionally, a clear decrease

of MMP-2 and MMP-9 expression levels was observed for all the cell lines under study.
Furthermore, we confirmed that engineered exosomes loaded with siMEK1 (iexo^{MEK1}) are not altered in their physical properties and overall integrity, being able to induce a powerful downregulation of *MEK1* expression. The *in vivo* experimental data demonstrated that iExo^{MEK1} induces a significant regression of the tumor size in relation to control conditions. Supported by the decrease of the number of recruited blood vessels, a reduction of metastasis and consequent inhibition of the formation of a second tumor was observed for iExo^{MEK1}-treated condition.

Conclusions

In summary, we have here clarified the role of MAPK/ERK suppression using a RNAi-based approach in different TNBC cell lines. Proliferation, migration and invasion impairments explained by the reversion of the EMT phenotype and the MMP-2/MMP-9 downregulation was verified upon *MEK1* downregulation. Additionally, a successful exosome-based platform was generated for the siRNA-targeting *MEK1* loading (iExo^{MEK1}), showing no changes in their physical properties and overall integrity in relation to unmodified exosomes. Ultimately, an *in vivo* tumor regression and angiogenesis decrease were observed after exosome-mediated *MEK1* downregulation, certifying this strategy as a novel promising approach towards TNBC.

Acknowledgments

This work was supported by by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469/2020 unit. Débora Ferreira is recipient of a fellowship supported by a doctoral advanced training (call NORTE-69-2015-15) funded by the European Social Fund under the scope of Norte2020 - Programa Operacional Regional do Norte. Débora Ferreira also acknowledges “Liga Portuguesa contra o cancro - Núcleo Regional do Norte (LPCC-NRN) “for her current fellowship.

References

[1] H. Sung, J. Ferlay, R. L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, F. Bray, *CA. Cancer J. Clin.* **2021**, *71*, 209.

[2] T. F. S. Mendes, L. D. Kluskens, L. R. Rodrigues, *Adv. Sci.* **2015**, *2*, 1500053.

[3] D. O’Reilly, M. Al Sendi, C. M. Kelly, *World J. Clin. Oncol.* **2021**, *12*, 164.

[4] M. N. Abad, S. Calabuig-Fariñas, M. L. de Mena, M. J. G. S. de Bremond, C. G. González, S. T. Martínez, J. Á. García-García, V. I. González-Cruz, C. C. Herrero, *https://doi.org/10.1177/1758835920986749* **2021**, *13*, DOI 10.1177/1758835920986749.

[5] Braicu, Buse, Busuioc, Drula, Gulei, Raduly, Rusu, Irimie, Atanasov, Slaby, et al., *Cancers (Basel).* **2019**, *11*, 1618.

[6] R. Yaeger, R. B. Corcoran, *Cancer Discov.* **2019**, *9*, 329.