FATTY MESS

Fat Diet-Induced Obesety in Melanoma Metastasis

Autores:[1] Joana Correia, [2]Raquel Costa, [1]Cristina Prudêncio, [2]Raquel Soares & [1]Pedro Coelho

[1]*Chemical Sciences and Biomolecules, School of Health, Polytechnic Institute of Porto; Department of Biomedicine, Faculty of Medicine, University of Porto*

Referência do Financiamento: Fundação para a Ciência e a Tecnologia, EXPL/SAU-NUT/0843/2021

Resumo

O melanoma é o cancro de pele mais agressivo, caracterizado por alto potencial metastático e baixa sobrevida. O excesso de peso e a obesidade foram identificados como fatores de risco para muitos tipos de cancro. No entanto, existem neoplasias em que a obesidade está associada a prognósticos mais favoráveis, o denominado "paradoxo da obesidade".

Dados preliminares obtidos pelo nosso grupo de investigação revelaram que a obesidade atua como uma faca de dois gumes no melanoma: potencializando o crescimento e a vascularização/angiogénese do tumor primário, mas simultaneamente diminuiu o potencial metastático, reduzindo o crescimento do tumor secundário.

Neste projeto exploratório, propomos explorar e aprofundar o impacto das dietas ricas em gordura e da obesidade na etiologia do melanoma.

Abstract

Melanoma is the most aggressive skin cancer characterized by a high metastatic potential and poor survival rate, leading to major morbidity/mortality. Overweight, obesity and diet composition have been identified as risk factors for many types of cancers. However, there are cancers where obesity is associated with favorable outcomes and this has been coined the "obesity paradox".

Preliminary data obtained by our research group disclosed paradoxical findings unveiling that obesity act as a two-edged sword in melanoma: potentiating primary tumor growth, and vascularity/angiogenesis, but at the same time decrease metastatic potential, thus preventing secondary tumor growth.

In this exploratory research project, we propose to further explore and deepen the impact that fat-rich diets and obesity partake in melanoma etiology.

Skin cancer is by far the most common of all cancers. Melanoma accounts for only about 1% of skin cancers but causes the large majority of skin cancer deaths. Several risk factors are known to contribute to melanoma development: sun/UV radiation, a family history of melanoma, high number of nevi, the degree of skin pigmentation, a suppressed immune system, among others.(Jiang et al., 2015) The risk of melanoma increases with age but, unlike other solid tumors, melanoma is not uncommon among those younger than 30.(Jiang et al., 2015) In fact, it's one of the most common cancers in young adults, accounting for the third-highest number of deaths across all cancers.(Apalla et al., 2017)

The incidence of cutaneous melanoma is consistently rising and obesity has been postulated as one of the causes for the increased incidence of melanoma. Obesity has been identified as risk factor for several types of cancer, however the association with melanoma incidence is not as strong.(Clement et al., 2017) Nevertheless, several reports showed positive associations between

adiposity and the risk of melanoma later in life suggesting that the increasing incidence of melanoma may be related, in part, with the enlarged obesity prevalence. Additionally, there is evidence supporting that feeding behavior and diet composition might be associated with melanoma etiology.(Karimi et al., 2016) Calorie restriction demonstrate a wide range of beneficial effects able to help prevent malignancies and increase the efficacy of cancer therapies. Concomitantly with melanoma expansion, the development of a supporting network of blood supply is vital to sustain tumor growth. Melanomas are highly vascularized tumors and angiogenesis has been correlated with poor overall survival and increased rate of relapse.(Kimura & Sumiyoshi, 2007) Contrastingly, the deregulated tumor microenvironment leads to a tortuous vascular network culminating in a low abundance of nutrients and oxygen within the tumor milieu. However, melanomas can generate an angiogenic-independent tumor microcirculation – socalled vasculogenic mimicry – to increase perfusion. Tumor cell-lined vascular networks sustain a redundant blood supply, with a viable blood flow between vasculogenic mimicry void spaces and the endothelial vasculature. The presence of these mimetic channels by the tumor itself stands a predictor of poor prognosis, but little is known about the biological relevance of this phenomenon.(Zhang et al., 2019) Adipocytes, obesity and fat-rich diets are well-known promoters of primary melanoma growth in preclinical models. High fat diet (HFD)-fed and overweight C57Bl/6 mice exhibit larger B16F10 tumors with increased microvessel density and higher-caliber capillaries. (Coelho et al., 2016; Fonseca et al., 2021) We have previously published reports on adipocytes ability to paracrine enhance B16F10 melanocytes proliferation, migration adhesion, invasion, antioxidant defenses and treatment resistance.(Coelho et al., 2016, 2017) Moreover, we demonstrated that adipocyte-released molecules induce melanoma cells to rearrange, on in vitro 3D cultures, into vessel-like structures typically reported for endothelial cells, endorsing the potential adipocyte secretome inducer effects of tumor vasculogenic mimicry.(Coelho et al., 2016) In fact, preliminary data obtained in a pilot study from our research group disclosed that not only the number and caliber of capillaries was elevated in overweight B16F10 tumor-bearing animals, but the increase in microvascular density was accompanied by a parallel higher number of vasculo-mimetic channels in vivo.(Fonseca et al., 2021) Therefore, we hypothesized that obesity could be exacerbating the vasculogenic mimicry microcirculation pattern in melanomas, endorsing a potential role of obesity towards the vasculogenic mimicry phenotype. Surprisingly, we also unveiled an unexpected antagonistic outcome of obesity in melanoma metastasis. Despite the increased volume of primary B16F10 tumors found in HFD-fed animals, the number of lung metastases in overweight animals injected with circulating

B16F10 cells was significantly lower than their lean counterparts. Obesity-associated changes apparently decrease B16F10 lung-tropism and metastatic potential thus restraining secondary tumor growth. Molecular characterization of adipose-associated serum factors revealed that HFD-fed mice exhibited remarkably large amounts of fibroblast growth factor (FGF)-2, FGF-21 and vascular endothelial growth factor (VEGF), with a concomitant overexpression of the FGFR-1 receptor by melanoma cells, highlighting possible new molecular mechanisms linking obesity and melanoma.(Fonseca et al., 2021)

The relative risk of obesity-associated cancer is now well established for 13 different malignancies.(Lauby-Secretan et al., 2016) Counterintuitively, adiposity appears to be protective and associated with favorable outcomes in other types of cancer, holding the "obesity paradox" hypothesis.(Pamoukdjian et al., 2019) For melanoma, numerous reports support fat-rich diets role as melanoma growth promoters. Actually, calorie-restriction is known to inhibit B16F10 cell tumorigenesis and slower melanoma growth while concomitantly enhancing pulmonar colonization in vivo. Our data point towards the inverse relationship, where HFD acts as a two-edged sword in melanoma: supporting primary tumor growth and vascularity, but at the same time decreasing melanoma metastatic spread.

Altogether, the strength of the association between adiposity and melanoma in human studies remains inadequate. It is our conviction that the "obesity paradox" hypothesis lies in between melanoma primary tumor versus secondary metastasis growth. Adiposity promotes cutaneous melanoma onset and growth, while protecting disease spread, therefore, undermining meta-analyses and other cohort studies estimates of the melanoma obesity-associated risk. Nevertheless, sufficient evidence exists to support ongoing and planned research studies to further acquire in-depth knowledge to attain a clearer understanding of this rationale.

Herein, and in accordance with our previous work, we aim to challenge the obesity paradox in malignant melanoma by taking advantage of the extensive expertise of the research team in the murine syngeneic B16F10 melanoma model, the most commonly used metastatic melanoma model for preclinical studies, and gathering together a team of researchers with solid knowledge in diet-induced obesity models, cancer metabolism and angiogenesis.

Our approach will improve the diet-induced obesity B16F10 melanoma model and comprises the development of a Luciferase and GFP dual-reporter melanoma cell line. Therefore, diet-induced obese C57Bl/6 mice inoculated with B16F10-Luc-GFP melanoma cells will be used as an in vivo model to

study the effects of obesity and fat-rich diets in melanoma development and metastasis. Briefly, we will transfect the B16F10 cells with Luciferase and GFP transgenes and create an auto-reporter melanoma cell line. Luciferase-expressing B16F10 cells will definitely enable in vivo noninvasive monitoring of primary melanoma growth and allow us to track the B16F10 cell metastatic spread. In fact, the C57Bl/6 - B16F10 melanoma model system is ideal to study by whole-body live imaging, given the high tissue contrast between tumor tissue and the surrounding parenchyma. Moreover, the GFP transgene will allow us straightforward visualization and analysis of tumors and metastasis in ex vivo tissues by conventional fluorescence microscopy and flow cytometry.

C57Bl/6 animals will be submitted to a diet challenge. The C57Bl/6 mouse model is a particularly good model mimicking the complications that are observed in human obesity. When fed ad libitum with an hyperlipidic diet, obesity, hyperinsulinemia, hyperglycemia and hypertension establish in these mice. Animals fed ad libitum with a normal-fat diet however, do not develop any metabolic abnormalities. Additionally, calorie restriction with a high-fat diet effectively attenuates obesity-related markers in rodents. Therefore, our diet-induced obesity model will include two diets with different fat contents that will be provided to two groups of animals: a HFD with 45% of kcal from fat and a NFD with 13% of fat by calories, respectively. To further improve the diet challenge, a third group of animals (ICD) will receive the HFD in a pair-fed system, guaranteeing they get the same daily amount of calories as their NFD counterparts. By examination of the pair-fed ICD animals, we will also be able to conclude whether the effect of diet in melanoma growth and metastization is due to excess consumption of calories or to an overload of dietary lipids. Afterwards, mice will be engrafted with B16F10-Luc-GFP cells to grow primary and metastatic melanomas in vivo. Subcutaneous inoculation of B16F10-Luc-GFP cells will induce primary skin melanomas, while intravenous injection of B16F10-Luc-GFP cells will directly introduce melanoma cells into the blood flow and lead to the development of distant metastases.

In vivo monitoring of orthotopic tumor development and disease dissemination will be performed by bioluminescent imaging of luciferase-expressing B16F10 cells, providing us with detailed information of tumour growth, volume and metastasis location/organ tropism of metastatic cells. Contrast-enhanced X-ray micro-computed tomography of tumor-bearing animals, will provide a highly detailed three-dimensional analysis of the vascular network within the primary melanomas and allow the quantification of the relative tumor blood perfusion and tumor neovascularization. Angiogenesis and vasculogenic mimicry will be evaluated by postmortem

analysis of tumor and metastasis sections. GFP expression by melanoma cells allied to immunofluorescence staining of CD31-positive endothelial cells will allow us to discriminate true endothelium-lined vessels from the tumor cell-derived vasculature. Overexpression of FGF-2, FGF-21 and VEGF, found in obese mice serum in our previous study, are known to be involved in angiogenesis and metabolic cues.(Coelho et al., 2016; Fonseca et al., 2021) In order to pinpoint the molecular bases of melanoma progression induced by the distinct diets, the expression of FGFR1 and FGFR2, as well as VEGFR1 and VEGFR2 will be investigated by RT-qPCR in both primary melanomas and distant metastasis. Receptors activity will further be examined by Western blotting using antibodies against total and phosphorylated (active) receptor forms. Depending on the findings, these signaling pathways will further be investigated, namely by examining Erk and Akt downstream effectors. The circulating levels of FGF-1, FGF-2 and FGF-21 and VEGF-A and -B ligands will be performed in mouse serum by ELISA.

We anticipate that, by the end of the project, further evidence supporting the obesity paradox in melanoma will be disclosed. It is expected that HFD-animals on one hand will bear considerably larger primary tumours with enhanced vascular networks but, on the other hand, will simultaneously exhibit lessened metastatic disease burdens. We also foresee that a calorie-restricted feeding regimen will reverse the deleterious effects of fat-rich diets in melanoma progression, hence ICD animals will present a primary disease burden closer to lean animals. Additionally, we expect to identify alterations in serum FGF/VEGF levels and FGFR/VEGFR expression and signalling that are distinctly modulated by HFD-feeding, which might contribute to further unravel the obesity paradox in melanoma.

Altogether, the main purpose of the current project is to help clarify the obesity paradox in melanoma, adding up to accumulating evidence and epidemiologic data and further strengthen the association between obesity and malignant melanoma progression. Nevertheless, this proposal might disclose a paramount dietary influence of fat-rich diets in melanoma progression and aggressiveness, concomitantly promoting beneficial and deleterious consequences with direct implications in disease prognosis, treatment outcomes and overall survival. Ultimately, this project will emphasize the importance of taking into consideration the role of adiposity in clinical practice to develop individualized treatment plans, with personalized therapeutic regimens, maximizing melanoma therapy success.

References

Apalla, Z., Nashan, D., Weller, R. B., & Castellsagué, X. (2017). Skin Cancer: Epidemiology, Disease Burden, Pathophysiology, Diagnosis, and Therapeutic Approaches. Dermatology and Therapy, 7(Suppl 1), 5–19. https://doi.org/10.1007/s13555-016-0165-y.

Clement, E., Lazar, I., Muller, C., & Nieto, L. (2017). Obesity and melanoma: could fat be fueling malignancy? Pigment Cell & Melanoma Research, 30(3), 294–306. https://doi.org/10.1111/pcmr.12584.

Coelho, P., Almeida, J., Prudêncio, C., Fernandes, R., & Soares, R. (2016). Effect of Adipocyte Secretome in Melanoma Progression and Vasculogenic Mimicry. Journal of Cellular Biochemistry, 117(7), 1697–1706. https://doi.org/10.1002/jcb.25463.

Coelho, P., Silva, L., Faria, I., Vieria, M., Monteiro, A., Pinto, G., Prudêncio, C., Fernandes, R., & Soares, R. (2017). Adipocyte Secretome Increases Radioresistance of Malignant Melanocytes by Improving Cell Survival and Decreasing Oxidative Status. Radiation Research, 187(5), 581–588. https://doi.org/10.1667/RR14551.1.

Fonseca, M., Soares, R., & Coelho, P. (2021). Lower melanoma pulmonary metastatic burden in obese mice: role of FGF-21. Melanoma Research. https://doi.org/10.1097/CMR.0000000000000781.

Jiang, A. J., Rambhatla, P. V., & Eide, M. J. (2015). Socioeconomic and lifestyle factors and melanoma: a systematic review. The British Journal of Dermatology, 172(4), 885–915. https://doi.org/10.1111/bjd.13500.

Karimi, K., Lindgren, T. H., Koch, C. A., & Brodell, R. T. (2016). Obesity as a risk factor for malignant melanoma and non-melanoma skin cancer. Reviews in Endocrine & Metabolic Disorders, 17(3), 389–403. https://doi.org/10.1007/s11154-016-9393-9.

Kimura, Y., & Sumiyoshi, M. (2007). High-Fat, High-Sucrose, and High-Cholesterol Diets Accelerate Tumor Growth and Metastasis in Tumor-Bearing Mice. Nutrition and Cancer, 59(2), 207–216. https://doi.org/10.1080/01635580701499537.

Lauby-Secretan, B., Scoccianti, C., Loomis, D., Grosse, Y., Bianchini, F., Straif, K., & International Agency for Research on Cancer Handbook Working Group. (2016). Body Fatness and Cancer--Viewpoint of the IARC Working Group. The New England Journal of Medicine, 375(8), 794–798. https://doi.org/10.1056/NEJMsr1606602.

Pamoukdjian, F., Aparicio, T., Canoui-Poitrine, F., Duchemann, B., Lévy, V., Wind, P., Ganne, N., Sebbane, G., Zelek, L., & Paillaud, E. (2019). Obesity survival paradox in cancer patients: Results from the Physical Frailty in older adult cancer patients (PF-EC) study. Clinical Nutrition, 38(6), 2806–2812. https://doi.org/10.1016/j.clnu.2018.12.011.

Zhang, Z., Imani, S., Shasaltaneh, M. D., Hosseinifard, H., Zou, L., Fan, Y., & Wen, Q. (2019). The role of vascular mimicry as a biomarker in malignant melanoma: a systematic review and meta-analysis. BMC Cancer, 19(1), 1134. https://doi.org/10.1186/s12885-019-6350-5.