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21876 | Development of a stable melanoma dual reporter cell line expressing Luciferase and GFP

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Background & Aim: Melanoma is the most aggressive and lethal form of skin cancer, with a high risk of metastatic spread [1]. Obesity is recognized as a risk factor for various types of cancer [2]. However, regarding melanoma, this association remains controversial. Obesity might act as a double-edged sword in melanoma, promoting primary tumour growth but at the same time limiting metastatic spread - the "obesity paradox" [3]. Herein, we aimed to create a stable murine B16F10 melanoma cell line expressing both firefly luciferase (Luc) and green fluorescent protein (GFP), which will later be engrafted into diet induced-obesity animal model for future *in vivo* studies. **Methods:** B16F10-Luc-GFP cells were generated by transfection with premade lentiviral particles, featuring a construct with Luc and GFP under a cytomegalovirus promoter and mediated by a F2A element. The antibiotic selection marker (puromycin) is expressed under a Rous sarcoma virus promoter. Afterwards, the transfected cells were selected with 1 µg/ml of puromycin. The clones with the highest levels of GFP-positive cells and GFP fluorescence were purified by two rounds of cell sorting and submitted to fluorescence and bioluminescence quantification, morphology, injury, BrdU incorporation, 7-AAD, and PI cell cycle assays and compared to the parental cell line. **Results:** B16F10-Luc-GFP were successfully generated, and both GFP fluorescence and D-luciferin bioluminescence are present and proportional to cell density. As expected, the parental cell line didn't display GFP or Luc activities. Moreover, transduced cells exhibit similar morphology, motility, proliferation, viability, and cell cycle progression as B16F10 cells. **Conclusions:** Altogether, the future engraftment of B16F10-Luc-GFP in obese mice, will improve melanoma research models, enabling the *in vivo* and *ex vivo* visualization of primary tumours and metastasis, providing a better understanding of the underlying molecular mechanisms, to clarify the "obesity paradox" in melanoma.

Keywords: Melanoma, B16F10, Obesity, GFP, Luciferase.

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