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Development FAP-Targeted Nanotherapy against Cancer-Associated Fibroblasts

Arianna Bonizzi[1], Marta Truffi[2], Leopoldo Sitia[1], Serena Mazzucchelli[1], Sara Negri[2], Luca Sorrentino[3], Marta Sevieri[1] and Fabio Corsi [1,2]

[1] Nanomedicine Laboratory, Department of Biomedical and Clinical Sciences "L. Sacco", University of Milan, 20157 Milan, Italy
arianna.bonizzi@unimi.it; leopoldo.sitia@unimi.it; serena.mazzucchelli@unimi.it; marta.sevieri@unimi.it;
[2] Istituti Clinici Scientifici Maugeri IRCCS, 27100 Pavia, Italy
marta.truffi@icsmaugeri.it; sara.negri@icsmaugeri.it; fabio.corsi@icsmaugeri.it
[3] Colorectal Surgery Unit, Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, 20133 Milan, Italy
luca.sorrentino@istitutotumori.mi.it;

Extended Abstract

In the recent years, cancer research has largely focused on biology of cancer-related stroma. The tumor microenvironment (TME) is a highly heterogeneous milieu consisting of different cell types and plays an important biological role in cancer development and progression. In particular, cancer-associated fibroblasts (CAFs) are the major cell population in the TME which support tumor growth, metastasis formation and induce drug resistance, through the production and secretion of variety of growth factors and cytokines, as well as extracellular matrix components [1]. A biological hallmark of CAFs is the selective expression of fibroblast activation protein (FAP), a membrane-bound serine protease of the dipeptidyl peptidase subfamily, not present in healthy tissues [2]. FAP is important for remodeling the extracellular matrix, plays a role in tumor invasion and immunosuppression, and it could be exploited as a target for innovative anticancer therapies against tumor stroma [3]. In this context, the rise of nano-approaches has offered new opportunities of treatment [4], promoting new strategies based on specific CAF-targeted delivery system by surface functionalization of the nanocages and allowing the encapsulation of cytotoxic drugs direct to these cells [5-6].

To this purpose, we developed a FAP-targeted nanotherapy against CAFs using H-ferritin nanocages (HFn-FAP) loaded with the pro-apoptotic drug Navitoclax (Nav), a Bcl-2 inhibitor able to induce apoptosis in CAFs [7].

CAFs were isolated from 4T1 breast tumors grown in mice [8] and characterized by Western blot, analyzing the expression of specific markers such as smooth muscle actin and FAP. Targeting and cytotoxic properties of engineered nanodrugs were investigated in cell culture in *vitro*. FAP+ CAFs displayed high binding capability with functionalized nanocages than FAP- cancer cells. This was correlated with surface expression of FAP in the selected cells. Nav-loaded functionalized nanocages (HNav-FAP) has been characterized and tested in comparison with non-functionalized HNav and free drug for in vitro efficacy on FAP+ cells. Raman spectroscopy, transmission electron microscopy, dynamic light scattering and UPLC/MS-MS results confirmed the characteristic structure of the nanocages and an effective nanoformulation of Nav. Indeed, as verified by apoptosis induction, BAX activation, and the caspase-dependent cleavage of the nuclear protein PARP-1, Nav is released inside the cells and maintains its native molecular activity in sensitive cells. The viability assay to evaluate the pharmacological activity of the nanoconstructs revealed that HNav-FAP induced remarkable cytotoxic effect in FAP+ cells in comparison to non-functionalized HNav and free drug administered at the same concentration. The increased activity of HNav-FAP was fully in accordance with a significantly higher drug release observed in FAP-overexpressing cells. Finally, we evaluated biodistribution and tumor targeting of HFn-FAP in a mouse syngeneic model of triple negative breast cancer after intravenous administration. In vivo preliminary results demonstrated that functionalized HFn is able to recognize CAFs and enhance intratumoral retention of the nanoparticles.

In conclusion, our findings suggest that FAP-targeted nanotherapy could be a promising strategy against CAFs to modulate the tumor microenviroment and provide new hope to fight the cancer.

References

[1] M. Truffi, L. Sorrentino, F. Corsi, "Fibroblasts in the Tumor Microenvironment," *Adv. Exp. Med. Biol*., 1234, 15–29, 2020.

[2] L. Xin, J. Gao, Z. Zheng, Y. Chen, S. Lv, Z. Zhao, C. Yu, X. Yang, R. Zhang, "Fibroblast Activation Protein-α as a Target in the Bench-to-Bedside Diagnosis and Treatment of Tumors: A Narrative Review," *Front. Oncol.,* 11:648187, 2021.

[3] M. Kraman, P. J. Bambrough, J. N. Arnold, E. W. Roberts, L. Magiera, J. O. Jones, A. Gopinathan, D. A. Tuveson, Douglas. T. Fearon, "Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein-alpha,"*Science*.," 330(6005):827-30, 2010.

[4] F. Mainini, A. Bonizzi, M. Sevieri, L. Sitia, M. Truffi, F. Corsi, S. Mazzucchelli, "Protein-Based Nanoparticles for the Imaging and Treatment of Solid Tumors: The Case of Ferritin Nanocages, a Narrative Review," *Pharmaceutics*.,13(12):2000, 2021.

[5] M. Truffi, S. Mazzucchelli, A. Bonizzi, L. Sorrentino, R. Allevi, R. Vanna, C. Morasso, F. Corsi, "Nano-Strategies to Target Breast Cancer-Associated Fibroblasts: Rearranging the Tumor Microenvironment to Achieve Antitumor Efficacy," *Int J Mol Sci*., 20(6): 1263, 2019.

[6] J. Guo, J. Guo, H. Zeng, Y. Chen, "Emerging Nano Drug Delivery Systems Targeting Cancer-Associated Fibroblasts for Improved Antitumor Effect and Tumor Drug Penetration," *Mol Pharm.*,17(4):1028-1048, 2020.

[7] D. Lagares, A. SantoS, P. E. Grasberger, F. Liu, C. K. Probst, R. A. Rahimi, N. Sakai, T. Kuehl, J. Ryan, P. Bhola, J. Montero, M. Kapoor, M. Baron, X. Varelas, D. J. Tschumperlin, A. Letai, A. M. Tager, "Targeted apoptosis of myofibroblasts with the BH3 mimetic ABT-263 reverses established fibrosis," *Sci. Transl. Med.*, 9(420):eaal3765, 2017.

[8] M. Truffi, L. Sitia, M. Sevieri, A. Bonizzi, M. A. Rizzuto, S. Mazzucchelli, F. Corsi, "Isolation of Primary Cancer-Associated Fibroblasts from a Syngeneic Murine Model of Breast Cancer for the Study of Targeted Nanoparticles," *The J Vis Exp.*, 171, 2021.