New kid on the block in theranostics: Glypican-3

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Primary liver cancer, of which hepatocellular carcinoma (HCC) represents the most common histologic subtype, is a leading cause of cancer death worldwide [1]. While localized HCC can be effectively managed with surgery, the therapeutic options for the advanced forms are still limited to systemic therapy (e.g. sorafenib or lenvatinib) or loco-regional approaches such as chemoembolization or radioembolization with ⁹⁰Y-loaded microspheres.

According to the international guidelines, the diagnosis of HCC in clinical practice is currently based on computed tomography (CT) or magnetic resonance imaging (MRI) and biopsy is only rarely performed. Recently published meta-analyses indicate that 3-phase CT and MRI present sensitivity of 63–76% and 77–90%, respectively, and specificity ranging 87–98% and 84–97% [2]. Nevertheless, misdiagnosis may occur, especially due to possible HCC atypical presentations, for example it has been reported that 10–20% of HCCs does not present arterial phase hyperenhancement, being detectable only in portal phase or in delayed images [3]. Furthermore, imaging-based diagnosis entails a lack of information concerning the biological features of HCC (i.e. grade of differentiation, cytomorphological abnormalities, number of mitotic divisions, etc.), thus running on a divergent track respect the so-called “personalized medicine”,

by which treatment should be tailored on the individual’s genetic and molecular characteristics [4].

Positron emission computed tomography (PET/CT) with ¹⁸F-fluorodeoxyglucose (¹⁸F-FDG), the most commonly used tracer in oncology, presents poor sensitivity for HCC diagnosis, most probably due to the elevated glucose-6-phosphatase enzymatic activity expressed in the healthy liver as well as in well-differentiated HCCs [5]. However, it has to be pointed out that ¹⁸F-FDG has a relevant impact on HCC prognostication, since only the more aggressive and less differentiated forms present increased glycolytic activity. On the other hand, PET/CT with ¹⁸F-fluorocholine (¹⁸F-cho), whose mechanism of uptake is strictly dependent on cell membrane turn-over, has been successfully applied for the visualization of well-differentiated HCCs. Therefore, dual tracer PET/CT with ¹⁸F-FDG/¹⁸F-cho has been proposed as imaging biomarker of HCC differentiation. Nevertheless, dual tracer PET/CT entails a meaningful dosimetric burden and its role in HCC work-flow needs to be further investigated with clinical studies [5].

In this scenario, novel approaches, especially those based on theranostic platforms, are warmly welcome. In recent years, glypican-3 (GPC3) has gained more and more attention from the scientific community for its diagnostic and therapeutic potential. Glypicans are a family of six membrane-bound heparan sulfate proteoglycans (GPC1 to GPC6) bound to cell membrane surface through a glycosylphosphatidylinositol (GPI) anchor, characterized by high similarity in size of the core protein, all exhibiting an N-terminal secretory signal peptide and a hydrophobic domain at the C-terminus [6]. Glypicans are strongly expressed during development and morphogenesis while they are repressed in adults. It has been recently demonstrated that GPCs are involved in tumor growth and development, through some not fully understood mechanisms [7]. Among GPCs’ family, GPC3 has emerged as the “star protein” for its recognized

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relevance in HCC diagnosis, prognostication and therapeutic management [7]. GPC3, in fact, has been found strongly overexpressed in HCC and soluble GPC3 has been demonstrated in the peripheral blood of HCC patients, while no GPC3 expression has been detected in healthy individuals, in fatty liver, in hepatitis or in cirrhotic subjects. Moreover, it has been found a correlation between the intensity of GPC3 expression in HCC histopathological samples and patients' clinical outcome. Remarkably, GPC3 is a cell-membrane associated biomarker, thus being particularly "exposed" and suitable for targeted theranostic approaches. Codrituzumab, a recombinant humanized monoclonal antibody (mAb) binding to human GPC3, resulted capable to elicit anti-tumoral antibody-mediated cytotoxic effect in vitro and in animal models.

Several efforts have been devoted to the development of radioimmunoconjugates suitable for GPC3-targeted imaging through immuno-PET. An attempt to obtain an immuno-PET agent has been performed by Carrasquillo and collaborators who labeled codrituzumab with the positron-emitting radionuclide iodine-124 (^{124}I) [8]. The radiocompound ^{124}I -codrituzumab resulted effective for the in vivo detection of GPC3 expression in HCC patients submitted to combined therapy with sorafenib plus codrituzumab. Nevertheless, the authors were not able to prove the usefulness of immuno-PET with ^{124}I -codrituzumab for predicting patients' response to treatment, since in the cohort of subjects included in the cited study ($n=7$), the combined use of codrituzumab plus sorafenib did not result in tumor response.

Another valuable research in the field was performed by Sham and collaborators, who synthesized a radiocompound, constituted by a GPC3-directed IgG1 labeled with the radionuclide zirconium-89 (^{89}Zr) [9]. This radioimmunoconjugate, namely ^{89}Zr - αGPC3 , showed highly specific binding to GPC3-expressing hepatoblastoma cell lines while it demonstrated minimal uptake in non-GPC3-expressing tumors. ^{89}Zr - αGPC3 was also tested in mice bearing GP3-positive orthotopic xenografts, injected with the radiocompound and subsequently submitted to microPET at one-day intervals from day 0 to day 7 post injection. Immuno-PET efficiently detected GPC3-positive xenografts: in animals with larger tumors (i.e. 4 mm) the highest tumor-to-background ratio was achieved on day 7, while in those bearing smaller

lesions (i.e. 1 mm) the highest tumor-to-background ratio was obtained on day 1.

The potential of immuno-PET relies, first of all, on its possible utilization to select patients who are more likely to benefit from GPC3-targeted therapies, such as vaccines and GPC3-directed immunotoxins. In this regard, it is worth mentioning the ongoing phase I study (<https://clinicaltrials.gov/ct2/show/NCT03884751>) aimed to evaluate the safety of Chimeric Antigen Receptor T Cells Targeting Glypican-3 (i.e. CAR-GPC3 T Cells) in patients with advanced HCC, progressing after standard systemic treatment. In the aforementioned clinical trial as well as in other GPC3-targeted therapies, the expression of GPC3 has to be assessed through hepatic biopsy before patients' enrollment. Nevertheless, biopsy represents an invasive procedure, not exempt from the risk of bleeding in hepatopathic patients, and is not suitable for evaluating heterogeneity in GPC3 expression among primitive tumor and distant metastases. In this perspective, GPC3-targeted immuno-PET might be applied for an in vivo and "whole body" assessment of GPC3 status in HCC patients enrolled for clinical trials. Furthermore, GPC3-directed theranostic approaches, based on the administration of radioimmunoconjugates labeled with beta- or alpha-emitting radionuclides, are under investigation. In this regard, Ludwig et al. labeled the GP3-specific IgG1 αGPC3 with the beta-emitter yttrium-90 (^{90}Y), through the chelating agent 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) [10]. Athymic mice bearing orthotopic xenografts of GP3-positive tumors (i.e. HepG2 human hepatoblastoma cells) were divided into 3 groups: group 1 and 2 were injected with the radiolabeled compound (^{90}Y -DOTA- αGPC3) at the dosage of 200 μCi and 300 μCi , respectively, while group 3 (control) was administered with the unlabeled compound (DOTA- αGPC3). Of note, mice treated with ^{90}Y -DOTA- αGPC3 at the dosage of 300 μCi experienced a significant decrease of alpha-fetoprotein (AFP) level, while an only minor decrease was observed in the group treated at 200 μCi and dramatically increased AFP values were registered in the control group. Figure 1 schematizes the potential theranostic applications of GPC3 for HCC management.

Although the aforementioned results are still preliminary and far from passing "from bench to bedside", GPC3-targeted radiotheranostics is worthy of attention from the nuclear medicine community for its potential as a powerful weapon against HCC.

Fig. 1 Schematization of GPC3-targeted theranostic approaches (left side of the scheme) for hepatocellular carcinoma (HCC) management. In the square box on the right side, GPC3 structure is represented: note the site of interaction between the radiolabeled MoAb (i.e. ^{124}I -codrituzumab) and the glycosylphosphatidylinositol (GPI)-anchor to the cell membrane (figure created with BioRender.com)

Author contributions LF and OS equally contributed to the article.

Compliance with ethical standards

Conflict of interest All the authors declare that they have no conflict of interest related to this spotlight.

References

1. Siegel RL, Miller KD, Jemal A (2019) Cancer statistics, 2019. *CA Cancer J Clin* 69:7–34. <https://doi.org/10.3322/caac.21551>
2. Jiang HY, Chen J, Xia CC et al (2018) Noninvasive imaging of hepatocellular carcinoma: From diagnosis to prognosis. *World J Gastroenterol* 24:2348–2362. <https://doi.org/10.3748/wjg.v24.i22.2348>
3. Elsayes KM, Chernyak V, Morshid AI et al (2018) Spectrum of pitfalls, pseudolesions, and potential misdiagnoses in cirrhosis. *AJR Am J Roentgenol* 211:87–96. <https://doi.org/10.2214/AJR.18.19781>
4. Schillaci O, Urbano N (2017) Personalized medicine: a new option for nuclear medicine and molecular imaging in the third millennium. *Eur J Nucl Med Mol Imaging* 44:563–566. <https://doi.org/10.1007/s00259-017-3616-5>
5. Filippi L, Schillaci O, Bagni O (2019) Recent advances in PET probes for hepatocellular carcinoma characterization. *Expert Rev Med Dev* 16:341–350. <https://doi.org/10.1080/17434440.2019.1608817>
6. Filmus J, Selleck SB (2001) Glypicans: proteoglycans with a surprise. *J Clin Invest* 108:497–501. <https://doi.org/10.1172/JCI13712>
7. Hara H, Takahashi T, Serada S et al (2016) Overexpression of glypican-1 implicates poor prognosis and their chemoresistance in oesophageal squamous cell carcinoma. *Br J Cancer* 115:66–75. <https://doi.org/10.1038/bjc.2016.183>
8. Carrasquillo JA, O'Donoghue JA, Beylertgil V et al (2018) I-124 codrituzumab imaging and biodistribution in patients with hepatocellular carcinoma. *EJNMMI Res* 8:20. <https://doi.org/10.1186/s13550-018-0374-8>
9. Sham JG, Kievit FM, Grierson JR et al (2014) Glypican-3-targeted ^{89}Zr PET imaging of hepatocellular carcinoma. *J Nucl Med* 55:799–804. <https://doi.org/10.2967/jnumed.113.132118>
10. Ludwig AD, Labadie KP, Seo YD et al (2019) Yttrium-90-labeled anti-glypican 3 radioimmunotherapy halts tumor growth in an orthotopic xenograft model of hepatocellular carcinoma. *J Oncol* 2019:1–7. <https://doi.org/10.1155/2019/4564707>

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