Unmet needs in developing nanoparticles for precision medicine

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Unravelling the -omic profiles of human diseases puts in perspective the vision of a post-omics era in which personalized treatment is standard clinical practice. Accumulated data have provided enough information to understand that diseases are no longer single units, but a variety of different disorders with distinct molecular backgrounds. In the post-omics era, the main question arising is how to deal with a new situation in which even high prevalent diseases are becoming a group of disease subsets with different treatment responses. This opens the concept of rare diseases because some subsets might not be more prevalent than a rare disease. A new era of the so-called precision medicine is envisioned in which tailor-made treatments for each single subset will be available. In this scenario, the real challenge becomes how to develop and made available new diagnostic systems to define these subsets and also, new targeted treatments. As expectations rise to improve disease outcomes and patient survival, new initiatives are taken to confront the upcoming social interest and its potential industrial impact.

Precision medicine has been successfully applied to some oncological diseases. Colorectal cancer patients are treated with Cetuximab, an antibody against EGFR, only when no KRAS mutations are detected [1]. Furthermore, in breast cancer patients, trastuzumab, an antibody directed against Herceptin-2, is only indicated if tumors show positive expression of herceptin-2 [2]. These

examples highlight the importance of using targeted therapies only when specific molecular scenarios are present. Adequate diagnostic methodologies are needed to better define the correct scenario and further, to provide tools for a fast follow-up of the treatment response. For this, specific biomarkers are needed to predict treatment outcomes in a more accurate manner. For instance, the recently introduced anti-PDL/anti-PDL1 immunotherapy is highly effective in 25% of patients with non-small-cell lung carcinoma. However, there are no biomarkers yet to predict which patients will benefit from this therapy [3]. Furthermore, specific disease molecular backgrounds or patient biological features might require alternative combined therapies and development of new ones (i.e., siRNA treatments). In order to succeed, there are still many challenges to solve to make precision medicine a reality and nanoparticles can certainly play a major role. The time for precision nanomedicine has come to stage.

Nanoparticles in diagnostic & theranostic applications

Because nanoparticles can be labeled with a long list of different tracers and molecules on their surface, including antibodies, aptamers and peptide recognition sequences, it is possible to target them against specific molecular backgrounds and proteins. Their potential use as new targeted imaging systems is considerably higher [4]. Quenched fluorochromes bound to nanoparticles with

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enzyme-sensitive peptide sequences have been proven successful to detect *in vivo* intratumoral activity of caspases as a sign of active apoptosis, a specific type of programmed cell death induced by chemotherapeutic drugs [5] or of metalloproteinases as indicators of disease progression in arthritic diseases [6]. The presence of these enzymes releases the fluorochromes that become unquenched and activate their signal emission as beacons. Also, labeled nanoparticles can be targeted against specific cell surface receptors and membrane proteins. RGD-labeled paramagnetic nanoparticles bind to the $\alpha v \beta 3$ integrin of tumor endothelial cells allow noninvasive detection of angiogenesis and vascular imaging using conventional MRI [7]. Nonetheless, even though the use of nanoparticles in imaging diagnostics and treatment monitoring is promising, there are still few unsolved needs to confront. Among them, crossing of the brain–blood barrier of the nanoparticles to ensure their use in neurological disorders in which the barrier remains intact, and to ensure adequate interactions with other biological membranes while preventing unwanted charge-dependent interactions with other proteins and receptors, in particular at the bloodstream. Also, to avoid mislocation to other tissues unrelated with targeted cells or tissues, such as sequestration by the reticulo-endothelial system in the liver and spleen, or to ensure enough amount of labeled nanoparticles to allow feasible and adequate readouts with current medical technologies, in particular when deep-tissue readings are required.

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Moreover, the possibility of combining imaging and treatment in a single nanoparticle is also a pursued challenge. In that context, nanoparticles with radionuclide-labeled octreotide and octreotate peptides against somatostatin receptors can target neuroendocrine tumors positive for somatostatin type-2 receptors, a useful example of theranostics by integrating both functions, imaging diagnostics and therapy [8]. Furthermore, the use of targeting moieties on their surface can increase treatment efficacy when drugs are also loaded inside nanoparticles as drug delivery vehicles. As we have recently shown, this is the case of paclitaxel-loaded nanoconjugates targeted against cancer stem cell surface receptors in breast and colon cancer models [9].

Drug delivery systems & the protein corona

However, the *in vivo* formation of a protein corona surrounding nanoparticles when these reach biological fluids

might hamper their binding abilities. The physicochemical characteristics of each nanoparticle define the way nanoparticles interact with their environment. Nanoparticles are capable to adsorb and desorb a mixture of distinct proteins from biological fluids into their surface, in a dynamic continuous adsorption and desorption process that strongly influences nanoparticles interactions and their intracellular fate [10,11]. As recently reported, protein corona can reduce *in vivo* cytotoxicity of MUC1-targeted core–shell chitosan nanoparticles loaded with SN-38 [12] and also, changes at the protein corona and surface hydrophilicity of nanoparticles using poloxamers correlate with nanoparticle uptakes by macrophages and cancer cells [13]. Interestingly, zwitterionic coatings of targeted nanoparticles can increase cellular uptakes, suggesting that surface modifications might help to overcome undesired modulatory effects of protein corona on cellular uptake and active targeting [14].

Drug delivery systems are intended to increase treatment efficacy while avoiding the drug release at the bloodstream to reduce adverse side effects. This is the case of Abraxane®, an albumin-based nanomedicine recently approved for the treatment of breast and pancreatic cancers [15,16]. However, to ensure efficacy, drug-loading optimization and adequate intracellular release are required, as well as to ensure blood stability and optimal pharmacokinetics. These are particularly relevant when the final goal is combination therapy or cytoplasmic release of siRNA sequences. Combined delivery of siRNAs targeting VEGF and kinesin spindle protein with lipid nanoparticles showed in a first-in-human combination trial strong tumor reduction and regression of liver metastasis in endometrial cancer patients [17]. However, it is still unsolved how to ensure a widespread delivery when cargos should be targeted to tissues in different places (i.e., lymph nodes) and specific organelles (i.e., mitochondria), or into a high number of cells widely distributed. Delivery must ensure adequate tissue/cell targeting and also that enough amounts of molecules/cargos are released at the right location to downregulate/activate specific molecular targets. In fact, in combination therapies, the relative rate of release of the delivered cargos and the order of their release might also influence final therapeutic outcomes and should be taken into account even at the preclinical level. Not to mention the need to avoid immunological reactions such as anaphylactic and allergic reactions, complement activation and the production of antibodies against nanoparticles (i.e., anti-PEG antibodies) [18].

Nanoparticles biodistribution & the enhanced vascular permeability effect

Moreover, because of their passive accumulation within tumors and certain tissues due to the enhanced vascular

permeability (EPR) characterizing newly formed vessels and inflammatory areas, nanoparticles can deliver higher amounts of drugs to target sites. Even though this concept has been recently challenged, some reported that nanoconjugates are able to deliver up to 10–15% of the injected dose at the tumor site *in vivo* [19]. This is highly relevant when one considers that the average rate of tumor accumulation for a free drug is 0.1% of the total intravenous injected dose. Nonetheless, in order to ensure an optimal accumulation of nanoparticles by EPR several issues should be considered. One of them is how to evaluate the level of EPR of an individual patient, because different EPR capacities will lead to different accumulation rates of nanoparticles. Treatment success and patient final outcome might deeply depend on this. Development of nanoparticles able to perform *in vivo* measurements of EPR are needed to improve the probabilities to succeed in precision nanomedicine approaches. This should be carefully considered when biodistribution and efficacy of nanoparticles are evaluated in *in vivo* models at preclinical level. Models should be well characterized for EPR to ensure their adequateness. Furthermore, patient treatments should be defined according to their EPR levels at target tissues, in particular, when EPR is a fundamental part of their treatment. A highly relevant issue for cancer patients and inflammatory-based diseases.

Even though optimization of new nanoparticle-based diagnostics and therapies is still far from desired, their use in precision medicine is becoming a fact as new developed systems reach clinical trials. Finding

specific reliable biomarkers is a must to stratify patients for precision medicine and to ensure adequate treatment follow-ups. However, this stratification is nowadays based on defining molecular background features of disease targets. Eventually, patient stratification should go further than this because treatment efficacy depends also in other features such as tissue/cell target location, biological barriers, patient immunological status and body homeostasis, which can influence PK/PD and ADME profiles of delivered drugs. These will certainly increase the number of different clinical scenarios. In this context, new drug delivery systems represent an opportunity to add active peptides, proteins, aptamers and nucleic acids, among others, to our limited portfolio of currently available drugs. The potentiality of using an arsenal of different nanoparticle-based systems to better suit a specific stratified profile undoubtedly opens the door to a future precision nanomedicine strategy.

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