Sensors International 1 (2020) 100025

Contents lists available at ScienceDirect

Sensors International

journal homepage: www.keaipublishing.com/en/journals/sensors-international

Protein corona-enabled serological tests for early stage cancer detection

Erica Quagliarini [a], Riccardo Di Santo [b], Daniela Pozzi [b], Giulio Caracciolo [b,*]

[a] Department of Chemistry, Sapienza University of Rome, P.le A. Moro 5, 00185, Rome, Italy
[b] Department of Molecular Medicine, Sapienza University of Rome, Viale Regina Elena 291, 00161, Rome, Italy

ARTICLE INFO

Keywords:
Early cancer detection
Protein corona
Nanotechnology

ABSTRACT

Early stage cancer detection is a major issue in current medicine. In recent years, nanotechnology is providing new alternatives for early diagnosis. Upon exposure to human plasma, several nanoparticle types (e.g. gold nanoparticles) are surrounded by a protein layer referred to as protein corona (PC). The PC changes the original identity of the nanoparticle conferring a new biological character. It is now accepted that slight variations in the composition of a protein source significantly varies the PC composition. Thus, nanomaterials incubated with plasma proteins of individuals with different physiological conditions generate PCs with different compositions. This gives rise to the new concept of personalized PC. Therefore, since protein patterns of subjects affected by certain pathologies differ from those of healthy ones, diagnostic technologies based on the evaluation of personalized PC could represent a fascinating opportunity for early disease detection. Herein, we review the concept of personalized PC along with recent advances on the topic, giving an overview of some innovative analytical approaches for early stage cancer detection.

1. Introduction

The World Health Organization (WHO) has reported that Cancer is the second leading cause of death globally, accounting with an estimated one in six deaths [1]. The incessant global growth of cancer deaths exerts an appalling strain on communities and health systems from physical and financial points of view. Thus, the increasing survival rate is a crucial point to rely on, by increasing efficient quality treatments and accessible early detection. In the past decades, the natural cancer progression has been acutely understood but the limited available technologies for its detection hindered the possibilities to get in deeper knowledge. Once cancer affects a tissue, tumours cells start growing uncontrollably and spreading other organs since the metastasizing process led the body to death [2]. Currently, several therapeutic modalities are handled to contrast the metastasis incurrence, usually a combination of surgery, chemotherapy, and radiotherapy. Nevertheless, all these approaches reserve different limitations especially associated with the surgical risk and unwanted side effects. Therefore, if cancer could be detected at the incipient stage and its progression early stopped, there will be higher possibilities to reduce the mortality associated to the disease. Up to now, several diagnostic techniques for early cancer detection have been developed ranging from the traditional ones as tissue biopsy or imaging techniques, i.e. ultrasound imaging (USI), magnetic resonance imaging (MRI), X-ray computed tomography (CT), to more recent techniques as molecular blood analysis for biomarker identification, generally through tandem mass spectrometry (MS/MS) [3]. Nonetheless, most of these approaches are expensive and time-consuming consequently a pressing urgency of non-invasive, cheap, and user-friendly methods of cancer detection is strongly required [4]. Herein, we give an overview of some innovative analytical approaches for early stage cancer detection, commenting on some selective examples. Particularly, we focus on new cancer diagnostic technologies based on the exploitation of the nano-bio-interactions between nanoparticles and blood samples.

2. "Personalized protein-corona" in cancer diagnosis nanotechnology

Recent advances in nanoparticle (NP) biotechnology have raised

* Corresponding author.
E-mail address: giulio.caracciolo@uniroma1.it (G. Caracciolo).

Production and hosting by Elsevier

https://doi.org/10.1016/j.sintl.2020.100025
Received 27 June 2020; Accepted 7 July 2020
Available online 24 July 2020
2666-3511/© 2020 The Authors. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

hopes in the discovery of new and promising diagnostic techniques. Thanks to the peculiar properties conferred by the nanoscale dimensions (ranging from 1 to 100 nm) NPs afford several tools to allow an earlier and more accurate diagnosis [5]. For this reason, nano-based systems are now being exploited for the capture of different blood biomarkers including proteins, DNA and exosomes that circulate in tumour cells. In fact, when a NP is immersed in a biological fluid its surface rapidly interacts with a series of biomolecules, leading to a dramatic change of its synthetic identity [6]. This bio-nano interfacing process generates a biomolecular shell, dynamic in nature and mostly composed of proteins. The protein layer, usually referred to as protein-corona (PC), is made of two components a hard corona, principally made of proteins strongly absorbed by NP surface, and a soft corona composed of those proteins less absorbed [7,8]. The hard corona is what the living machinery firstly 'sees' and, therefore, it influences most of the biological responses. Recently, it was proved that PC composition is shaped by several factors such as NPs surface properties (e.g. size, shape, surface chemistry and charge), biological media properties (e.g. protein concentration and protein source) and experimental conditions (e.g. incubation time and temperature) [9]. A radical point was the discovery made by Mahmoudi and co-workers that the PC is personalized and influenced by the changes in concentration and structure of individual plasma proteins [10]. Particularly, they demonstrated that various diseases may cause alterations in plasma proteome or proteins conformation leading to drastic changes in PCs' identity. Thus, different pathologies cause formation of different PCs, hence the novel concept of "personalized PC" (Fig. 1 panel A). This disclosure is momentous for cancer diagnosis and prognosis as protein alteration is a predictive sign of the tumour progression since human proteome develops in time as a function of the disease evolution. In a series of seminal papers [11–13], Huo and coworkers developed PC-based approaches for prostate cancer detection. This technique detects the target analytes (e.g. tumour-associated auto-antibodies) by monitoring gold nanoparticle size change upon specific binding with the target molecules using dynamic light scattering.

Therefore, PC analysis for early cancer detection is a valid investigation strategy, especially if compared to the conventional blood analyses that, in most cases, do not appreciate variations in the circulating level of proteins [14]. In fact, protein alterations, that are generally small, can be easily detected exploiting the NPs ability to concentrate poorly abundant plasma protein that has an affinity for NP surface. In this way, PC acts as nano-concentrator of those proteins and allows the detection of minor changes even at the very early stage of the disease [13]. PC-based technologies for early detection are currently investigated for various cancers. Generally, in a PC analysis protocol, a specific class of NPs is incubated with plasma or serum patients to allow protein interactions with NP surface [15]. This step is followed by the separation of unbounded proteins and the isolation of the PCs from the NPs surface. Finally, the isolated PCs is analysed through different detection methods. Fig. 1 (panel B) shows a representative diagnosis protocol based on the PC evaluation. There are several determining factors to take in account in the choice of a cancer diagnosis approach of specific tumour classes i) the nano-sized material, according to its surface properties, must express high affinity for blood proteins ii) the experimental conditions must be adapted to avoid loss of proteins that are essential to discern between donor classes iii) the PC detection technique must have high sensitivity and specificity and must be rapid and cheap as much as possible. The choose of the NP is correlated to the right physicochemical properties that allow the best interactions with proteins during the incubation with the biological fluid [16]. The nature of the interaction mainly depends on the charge of the NP surface (positive, negative or zwitterionic) and its hydrophobicity/hydrophilicity. For instance, gold NPs have been largely employed for their potential in disease therapeutics especially for their high biocompatibility, easy synthesis/functionalization and high specificity in tissue targeting. Arvizo et al. investigated the PCs derived from the incubation of gold NPs with clinically relevant biological fluid testing whether the variation of NP surface charge allows enrichment of relevant but low abundant protein biomarkers [17]. The modulation of the gold NP surface charge was crucial for determining a possible therapeutic target for ovarian cancer. Currently, most of the applications in nano-biomedicine are focused on the exploitation of liposomes especially in the field of cancer diagnosis. The possibility to coordinate distinct lipid formulations to generate a wide range of multi-component shells promotes the analysis of different liposome-blood protein interactions, enabling to get the liposome PC that is appropriate to the own diagnostic aim or the one that best discerns between different donor classes. About that, we employed a liposomal formulation made of specific lipids that constitute the basis of the amphotericin B agent, an ideal model for studying the liposomal-blood proteins interactions [18]. The work aimed to understand if the liposome-PC composition was altered when derived from the incubation of pancreatic ductal adenocarcinoma (PDAC) plasma donors. In the end, we confirmed that the liposome exposure to the patient plasma significantly modified the composition of the PC with respect to the healthy one. These results lay the foundation to an easy diagnostic approach for cancer that exploits the potentiality of the PC modulation leading hopes to the advancement at the clinical phase. Nevertheless, liposomes suffer from long-term stability issues and batch to batch variations causing severe concerns about the transition at the clinical practice [19]. For this reason, in recent years alternative nanomaterials are being investigated to contrast these limits. Among them, graphene oxide has been largely studied in diagnostic research. This material boasts of low-cost production, dispersibility in water solvents, high surface area that is also enriched of oxygen groups with high reactivity for linking proteins [20]. One of its advantages, if compared to its reduction form, is the lower affinity that its surface has for albumin, the most abundant blood protein [21,22]. This is a key point as it consents to have preferentially bonds with proteins that are present at lower concentration in blood, allowing a more sensitive differentiation between individual classes. Thus, many studies were focused on the interaction and functionalization of its surface with plasma proteins. As for our work, we used graphene oxide to generate a blood test based on PC characterization for PDAC detection by incubating graphene oxide nanoflakes with plasma samples derived from healthy subjects and PDAC diagnosed ones [23]. PDAC was chosen as a model of high mortality disease with low survival rate since its diagnosis occurs generally when the cancer is already metastatic [24]. The results confirmed again the high power of PC in differentiating healthy from no healthy subjects even at the early stage of the tumour. However, the choice of NP is only one of the factors that must be evaluated in the development of a PC-based diagnosis protocol. As mentioned before, the exposure conditions as incubation time, temperature, biological media concentration strongly affect the composition of the resulting PCs [16]. For instance, protein adsorption is controlled by the exposure time of the NPs to the media. Indeed, it has been demonstrated that PCs of silica and polystyrene NPs form rapidly in less than 30 s maintaining the same composition over time [25]. Long duration of incubation time enhances the total protein amount adsorption with a continuous binding exchange between the same protein over time. Temperature also alters protein-NP interactions. Incubation temperature higher than the physiological temperature (37 °C) may cause modifications on secondary and tertiary protein structures that result in different bindings on NP surface [16]. After All, in recent years some overlooked factors have been disclosed as the impact of the protein concentration, protein gradient and protein source on the PC composition [26]. In this regard, one of our work was centred on the effects that graphene oxide lateral size and human plasma protein concentration have on PC composition and personalization [27]. We demonstrated that the lateral size does not influence the graphene oxide-PC composition whereas the protein concentration has an extraordinary impact. Notably, at low protein concentration, PC of pancreatic cancer patients was radically different from that of healthy donors. These results suggested that concentration of plasma should be properly regulated to emphasize PC differentiation from healthy and no healthy donors. This may be an important preliminary step to develop more sensitive and sensible technologies based on PC characterization.

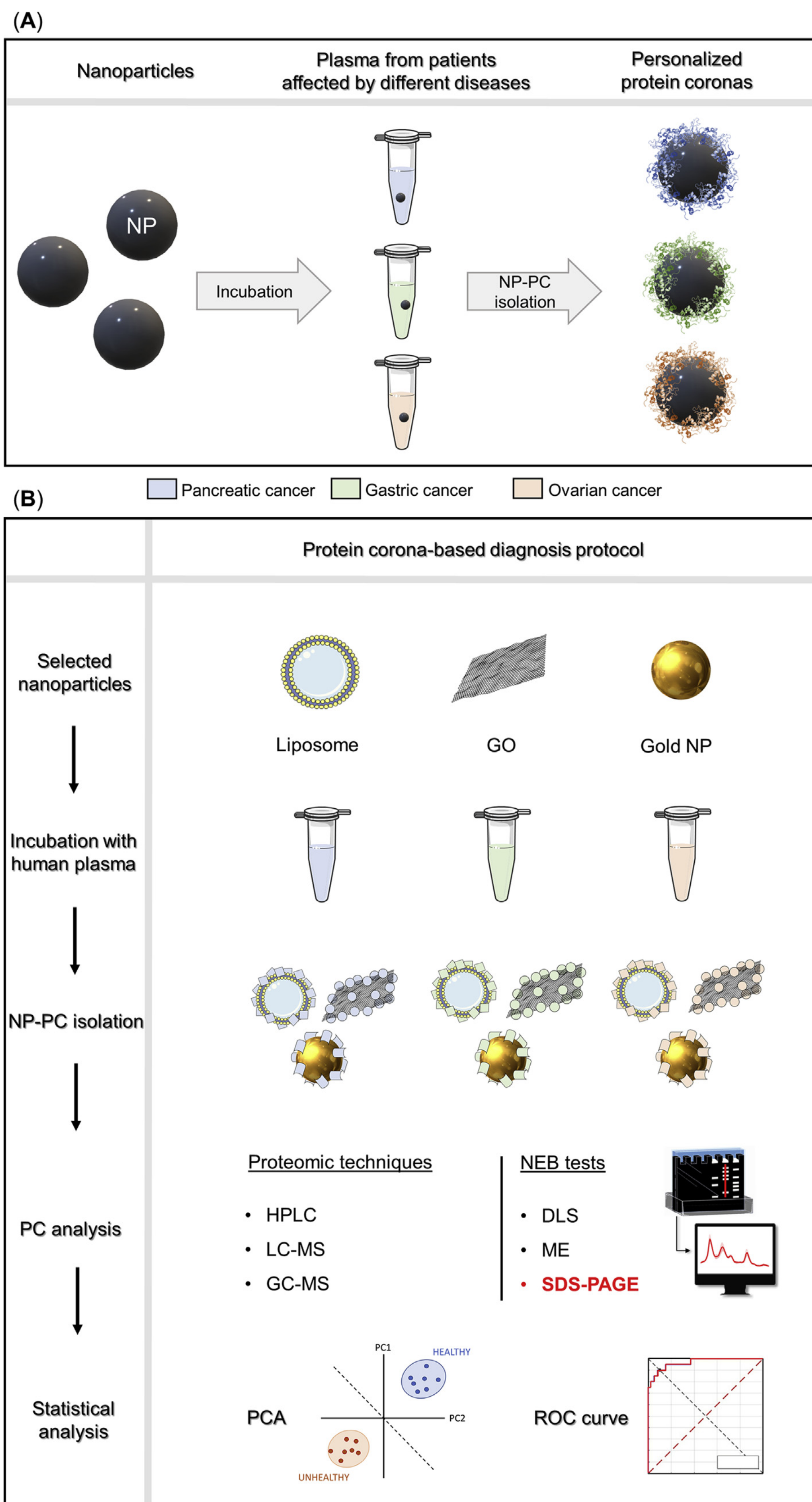

Fig. 1. Personalized protein corona-enabled blood test. (**A**) Protein coronas (PCs) obtained upon incubation of nanoparticles (NPs) with plasma derived from patients with different diseases (e.g. pancreatic, gastric and ovarian cancer) are personalized. (**B**) Schematic representation of the personalized PC workflow analysis for diagnostic purpose. NPs of different materials such as liposomes, graphene oxide (GO) and gold NPs are exposed plasma from different individuals. Incubation leads to personalized protein coronas that are isolated from NPs and thoroughly characterized by proteomics-techniques allowing to identify and quantify plasma proteins enriching personalized Ps. On the other side, nanoparticle-enabled blood (NEB) tests allow to distinguish patients affected by specific diseases with high sensitivity and specificity. The NEB test is based on typical outputs of benchtop techniques such as dynamic light scattering (DLS), micro-electrophoresis (ME) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3. Characterization of personalized protein corona

Currently, most of the technologies for PC analysis are based on proteomics [28]. High-performance liquid chromatography (HPLC), radioimmunoassay, nuclear magnetic resonance (NMR) spectroscopy, liquid chromatography and gas chromatography-mass spectrometry (LCMS and GCMS) or enzyme immunoassay can be used to discover metabolic alteration related to pathological conditions (metabolic biomarkers) [29]. Notably, mass spectrometry (MS) represents the basis of most proteomic experiments [30]. The ability of mass spectrometry (MS) to identify minor changes in the human proteome allows the discovery of single protein biomarker and to get information about the composition and function of PC. Although these approaches boast of high sensitivity and specificity allowing to analyse complex protein mixtures during a single experiment, their use is still limited as it requires laborious and costly procedures that are not appropriate for large scale production. In fact, WHO states that experimental procedures for cancer screening and detection must satisfy ASSURED (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free and Deliverable to end-users) criteria. As a consequence, researchers are combining low-resolution benchtop techniques to create adequate, safe, and inexpensive screening procedures. In this regard, NP-enabled blood (NEB) tests are emerging as new fast and cheap technology for PC characterization in the field of early cancer detection [31]. These tests are based on the characterization of NP-PC in terms of size, surface charge and composition through simple techniques as dynamic light scattering (DLS), micro electrophoresis (ME) and one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (1D-SDS-PAGE) [32]. In contrast to MS, the force factor of a NEB test is the possibility to obtain an overall evaluation of the 1D protein pattern. This allows a differentiation between donor groups that relies on the global and systematic alteration of several proteins when the type of NP, cancer or stage changes. Among the techniques included in the NEB tests, 1D-SDS-PAGE is certainly the most appropriate for the protein patterns differentiation [28]. 1D-SDS PAGE separates complex protein mixtures by molecular weight (MW), thanks to an electric field. Its use is specially directed to comparison purposes since it does not rely on quantitative outcomes but rather on qualitative ones. Indeed, it may allow a simultaneous resolution and distinction of several PCs from different NP incubation conditions. In a recent work, we developed a PC-based NEB test to distinguish patients affected by meningeal tumours from healthy subjects [33]. Currently, there is no method to detect brain tumour until the incoming of the symptomatic stage. Imaging techniques improve the neuro-radiological diagnosis, but they are expensive and not much specific, thus there is a pressing need for non-invasive methods to diagnose carcinoma of the central nervous system. In our investigation blood samples of 25 meningeal tumours and 20 healthy subjects were analysed by SDS-PAGE. As for most of NEB tests based on SDS-PAGE characterization, a semi-quantitative analysis of the intense bands of the gel is made by means of dedicated software and methodologies. In this work, we characterized three major protein bands located at different MW (i.e. 150, 50 and 25 kDa). The lane intensity profiles were divided into four regions and analysed. The major differences in PC intensity were found at MW range between 75 and 100 kDa. Thus, for a complete PC characterization, MS analysis was performed to identify the proteins related to that specific region, finding six potential protein biomarkers for CNS cancer. This result was notable since it opened the door for a new diagnostic approach for CNS cancer. In a similar study, we investigated the correlation between variations of plasma protein in breast, gastric and pancreatic cancer incubated with clinically approved AmBisome-like liposomes, by means of SDS-PAGE [34]. The strength of this work was the employment of semi-quantitative densitometry for analysing the protein profiles related to the different pathologies. The densitometry analysis was performed on the protein band intensity related to the hard coronas of the liposomes incubated with plasma samples. We firstly get an insight into the total protein content related to the PCs derived from the same plasma by measuring the total lane intensity of the protein recovered by liposome PCs. We obtained that the intensity of the protein profiles differs between each sample. Notable intensity differences were observed in four major bands located at MW˜110, 90, 75 and 37 kDa. The same approach was used for the PCs derived from each pathology. The trend was almost the same, with evident differences in lane intensity between each pathology in the same MW regions. In final, we compared the total lane intensity of the PCs derived from the unhealthy subjects with the ones derived from healthy ones. We noticed that the total lane intensity was substantially higher for pancreatic cancer with the four selected bands much more intense. These outcomes confirmed that the PC is personalized and an SDS-PAGE analysis on PC can be exploited for the differentiation between donor classes.

4. Statistical analysis of data

The evaluation of a diagnostic test is a matter of concern in modern medicine. A PC-based NEB test, principally founded on SDS-PAGE gel profiles, requires the analysis of a complex data set since there are many variables factors that must be taken into account (i.e. integral areas of 1D protein patterns, size and zeta potential of the NP etc.). Thus, the principal aim of the statistical analysis is to find a relation between each data extrapolating the possible covariance values and put them in a covariance matrix. Certainly, the information contained in the matrix must be translate in consistent data. In this way, Principal Component Analysis (PCA) is a powerful dimensionality reduction technique that precisely helps in extracting the relevant parameters from the big data of the covariance matrix [35]. Indeed, by means of PCA, it is possible to create new linear combinations, orthogonal by construction (principal component scores), of the original data, each representing an independent aspect of the data set (e.g. it may group the relative abundance of proteins in a specific MW region to simplify the discrimination between healthy volunteers and unhealthy ones). In this way, the meaningful part of the information is kept in the principal components and the rest is considered noise. In an already mentioned work, we used PCA to establish the effectiveness of a liposome-based NEB test used to discriminate pancreatic cancer affected patients from healthy volunteers [18]. The PCA showed that the two donor classes were strongly separated by the two principal components (derived from SDS-PAGE analysis of four integral areas calculated from the total lane intensity profiles in four regions of MW) and, in particular, the NEB test revealed 100% specificity and 85% sensitivity with total correctness of 88%.The power of the NEB test was then compared with the one get by a conventional blood test. The PCA on 24 haematological parameters that described patients' condition exhibited lower specificity and sensitivity with total correctness of 52%. Nevertheless, PCA is only one of the statistical tools that can be employed for PC data analysis. Namely, the receiver operating characteristic (ROC) curve can also be used to evaluate the diagnostic accuracy of a NEB-like test [36]. ROC analysis is generally used to quantify how accurately medical diagnostic tests can discriminate between two patient states. The curve is founded on a separator scale where the outputs for the patient state (generally healthy and unhealthy) form a pair of overlapping distributions. The complete distinction of the two distributions means a perfectly discriminating test and the sensitivity and specificity of the test are measured by the area under the curve (AUC) that describes the inherent validity of the diagnostic test. For instance, in a recent investigation on the characterization of the graphene oxide PCs, we used ROC curve to measure the correctness of the NEB test applied on the protein profiles obtained by 1D SDS-PAGE of the PCs of 50 volunteers, half of them healthy and half pancreatic cancer affected [23]. We obtained an area under the curve (AUC) of 0.96 and a distinction between the two donor classes with a sensitivity of 92%.

5. Conclusion

In conclusion, the characterization of the personalized PC, i.e. the

dynamic protein shell that coats the NP after exposure of patients' plasma, through benchtop techniques as 1D SDS-PAGE is introducing the possibility of cancer identification at very early stages. A similar test does not reveal the biomarkers associated to the disease but permits the detection of global changes in the protein patterns at the early stage of tumour or a therapeutic treatment. This is an innovative approach, especially if compared to the conventional blood test that usually cannot detect the slight alterations in the circulating levels of single proteins. Furthermore, the selective employment of 1D SDS PAGE for PCs characterization and differentiation meets the ASSURED criteria stated by WHO for cancer detection and screening thus, its application may help in the development of optimized NEB test with more sensitivity and specificity.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The research leading to these results has received funding from AIRC under IG 2017 - ID. 20327 project – P.I. Caracciolo Giulio.

References

[1] F. Bray, A. Znaor, P. Cueva, A. Korir, R. Swaminathan, A. Ullrich, S.A. Wang, D.M. Parkin, Planning and Developing Population-Based Cancer Registration in Low-And Middle-Income Settings, IARC (International Agency for Research on Cancer), 2014.
[2] D.F. Quail, J.A. Joyce, Microenvironmental regulation of tumor progression and metastasis, Nat. Med. 19 (2013) 1423.
[3] R.M. Sallam, Proteomics in Cancer Biomarkers Discovery: Challenges and Applications, Disease markers, 2015, p. 2015.
[4] D. Caputo, M. Cartillone, C. Cascone, D. Pozzi, L. Digiacomo, S. Palchetti, G. Caracciolo, R. Coppola, Improving the accuracy of pancreatic cancer clinical staging by exploitation of nanoparticle-blood interactions: a pilot study, Pancreatology 18 (2018) 661–665.
[5] J.T. Cole, N.B. Holland, Multifunctional nanoparticles for use in theranostic applications, Drug Del. Trans. Res. 5 (2015) 295–309.
[6] G. Caracciolo, Liposome–protein corona in a physiological environment: challenges and opportunities for targeted delivery of nanomedicines, Nanomed. Nanotechnol. Biol. Med. 11 (2015) 543–557.
[7] A. Bigdeli, S. Palchetti, D. Pozzi, M.R. Hormozi-Nezhad, F. Baldelli Bombelli, G. Caracciolo, M. Mahmoudi, Exploring cellular interactions of liposomes using protein corona fingerprints and physicochemical properties, ACS Nano 10 (2016) 3723–3737.
[8] G. Caracciolo, F. Cardarelli, D. Pozzi, F. Salomone, G. Maccari, G. Bardi, A.L. Capriotti, C. Cavaliere, M. Papi, A. Laganà, Selective targeting capability acquired with a protein corona adsorbed on the surface of 1, 2-dioleoyl-3-trimethylammonium propane/DNA nanoparticles, ACS Appl. Mater. Interfaces 5 (2013) 13171–13179.
[9] D. Pozzi, G. Caracciolo, A.L. Capriotti, C. Cavaliere, G. La Barbera, T.J. Anchordoquy, A. Laganà, Surface chemistry and serum type both determine the nanoparticle–protein corona, J. Prot. 119 (2015) 209–217.
[10] C. Corbo, R. Molinaro, M. Tabatabaei, O.C. Farokhzad, M. Mahmoudi, Personalized protein corona on nanoparticles and its clinical implications, Biomater. Sci. 5 (2017) 378–387.
[11] Q. Huo, J. Colon, A. Cordero, J. Bogdanovic, C.H. Baker, S. Goodison, M.Y. Pensky, A facile nanoparticle immunoassay for cancer biomarker discovery, J. Nanobiotechnol. 9 (2011) 20.
[12] Q. Huo, S.A. Litherland, S. Sullivan, H. Hallquist, D.A. Decker, I. Rivera-Ramirez, Developing a nanoparticle test for prostate cancer scoring, J. Transl. Med. 10 (2012) 44.
[13] T. Zheng, N. Pierre-Pierre, X. Yan, Q. Huo, A.J. Almodovar, F. Valerio, I. Rivera-Ramirez, E. Griffith, D.D. Decker, S. Chen, Gold nanoparticle-enabled blood test for early stage cancer detection and risk assessment, ACS Appl. Mater. Interfaces 7 (2015) 6819–6827.
[14] M.J. Hajipour, S. Laurent, A. Aghaie, F. Rezaee, M. Mahmoudi, Personalized protein coronas: a "key" factor at the nanobiointerface, Biomater. Sci. 2 (2014) 1210–1221.
[15] M.P. Monopoli, A.S. Pitek, I. Lynch, K.A. Dawson, Formation and characterization of the nanoparticle–protein corona, in: Nanomaterial Interfaces in Biology, Springer, 2013, pp. 137–155.
[16] B.-J.L. Van Hong Nguyen, Protein corona: a new approach for nanomedicine design, Int. J. Nanomed. 12 (2017) 3137.
[17] R.R. Arvizo, K. Giri, D. Moyano, O.R. Miranda, B. Madden, D.J. McCormick, R. Bhattacharya, V.M. Rotello, J.-P. Kocher, P. Mukherjee, Identifying new therapeutic targets via modulation of protein corona formation by engineered nanoparticles, PloS One 7 (2012).
[18] D. Caputo, M. Papi, R. Coppola, S. Palchetti, L. Digiacomo, G. Caracciolo, D. Pozzi, A protein corona-enabled blood test for early cancer detection, Nanoscale 9 (2017) 349–354.
[19] N.V. Dhandapani, A. Thapa, G. Sandip, A. Shrestha, N. Shrestha, R.S. Bhattarai, Liposomes as novel drug delivery system: a comprehensive review, Int. J. Res. Pharm. Sci. 4 (2013) 187–193.
[20] V. Palmieri, G. Perini, M. De Spirito, M. Papi, Graphene oxide touches blood: in vivo interactions of bio-coronated 2D materials, Nanoscale Horizons 4 (2019) 273–290.
[21] V. Palmieri, M.C. Lauriola, G. Ciasca, C. Conti, M. De Spirito, M. Papi, The graphene oxide contradictory effects against human pathogens, Nanotechnology 28 (2017) 152001.
[22] V. Palmieri, M. Papi, C. Conti, G. Ciasca, G. Maulucci, M. De Spirito, The future development of bacteria fighting medical devices: the role of graphene oxide, Expet Rev. Med. Dev. 13 (2016) 1013–1019.
[23] M. Papi, V. Palmieri, L. Digiacomo, F. Giulimondi, S. Palchetti, G. Ciasca, G. Perini, D. Caputo, M.C. Cartillone, C. Cascone, Converting the personalized biomolecular corona of graphene oxide nanoflakes into a high-throughput diagnostic test for early cancer detection, Nanoscale 11 (2019) 15339–15346.
[24] L. Zhang, S. Sanagapalli, A. Stoita, Challenges in diagnosis of pancreatic cancer, World J. Gastroenterol. 24 (2018) 2047.
[25] S. Tenzer, D. Docter, J. Kuharev, A. Musyanovych, V. Fetz, R. Hecht, F. Schlenk, D. Fischer, K. Kiouptsi, C. Reinhardt, Rapid formation of plasma protein corona critically affects nanoparticle pathophysiology, Nat. Nanotechnol. 8 (2013) 772.
[26] G. Caracciolo, D. Pozzi, A.L. Capriotti, C. Cavaliere, P. Foglia, H. Amenitsch, A. Lagana, Evolution of the protein corona of lipid gene vectors as a function of plasma concentration, Langmuir 27 (2011) 15048–15053.
[27] R. Di Santo, L. Digiacomo, E. Quagliarini, A.L. Capriotti, A. Laganà, R. Zenezini Chiozzi, D. Caputo, C. Cascone, R. Coppola, D. Pozzi, Personalized graphene oxide-protein corona in the human plasma of pancreatic cancer patients, Front. Bioeng. Biotechnol. 8 (2020) 491.
[28] A.L. Capriotti, G. Caracciolo, C. Cavaliere, V. Colapicchioni, S. Piovesana, D. Pozzi, A. Laganà, Analytical methods for characterizing the nanoparticle–protein corona, Chromatographia 77 (2014) 755–769.
[29] A.L. Capriotti, G. Caracciolo, G. Caruso, C. Cavaliere, D. Pozzi, R. Samperi, A. Laganà, Analysis of plasma protein adsorption onto DC-Chol-DOPE cationic liposomes by HPLC-CHIP coupled to a Q-TOF mass spectrometer, Anal. Bioanal. Chem. 398 (2010) 2895–2903.
[30] A.L. Capriotti, G. Caracciolo, G. Caruso, C. Cavaliere, D. Pozzi, R. Samperi, A. Laganà, Label-free quantitative analysis for studying the interactions between nanoparticles and plasma proteins, Anal. Bioanal. Chem. 405 (2013) 635–645.
[31] M. Papi, G. Caracciolo, Principal component analysis of personalized biomolecular corona data for early disease detection, Nano Today 21 (2018) 14–17.
[32] D. Caputo, G. Caracciolo, Nanoparticle-enabled blood tests for early detection of pancreatic ductal adenocarcinoma, Canc. Lett. 470 (2020) 191–196.
[33] M. Papi, V. Palmieri, S. Palchetti, D. Pozzi, L. Digiacomo, E. Guadagno, M. del Basso De Caro, M. Di Domenico, S. Ricci, R. Pani, Exploitation of nanoparticle-protein interactions for early disease detection, Appl. Phys. Lett. 114 (2019) 163702.
[34] V. Colapicchioni, M. Tilio, L. Digiacomo, V. Gambini, S. Palchetti, C. Marchini, D. Pozzi, S. Occhipinti, A. Amici, G. Caracciolo, Personalized liposome–protein corona in the blood of breast, gastric and pancreatic cancer patients, Int. J. Biochem. Cell Biol. 75 (2016) 180–187.
[35] H. Abdi, L.J. Williams, Principal component analysis, Wiley Interdis. Rev.: Comput. Stat. 2 (2010) 433–459.
[36] K. Hajian-Tilaki, Receiver operating characteristic (ROC) curve analysis for medical diagnostic test evaluation, Caspian J. Int. Med. 4 (2013) 627.