Extracellular Vesicles for Cancer diagnosis

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Early diagnosis is required to improve clinical outcomes in cancer patients. The invasive procedures required for the majority of currently available diagnostic tools represent the foremost drawback. Recently Extracellular vesicles (EVs) have gained interest as potential biomarkers alone or in combination with currently available tumour markers. In this review we discuss the impact of EVs as non-invasive or minimally invasive approaches for clinical application in the central nervous system, head and neck, lung, and gastrointestinal cancers.

extracellular vesicles cancer cancer diagnostic biomarkers liquid biopsy

1. Introduction

Despite scientific advances in cancer biology, diagnostic techniques, and new therapeutic approaches, the overall survival (OS) of cancer patients remains largely unfulfilling. Some cancers hold low mortality, particularly prostate and thyroid cancer, while others, such as central nervous system (CNS), breast, lung, oesophageal, gastric, and pancreatic cancers, are characterized by worse outcomes, and their metastatic disease is generally fatal [1][2][3][4][5]. Liquid biopsy refers to the detection of cancer cells and/or cancer cell products/derivatives in body fluids for diagnosis, monitoring, treatment efficacy, and prognosis [6]. Among new cancer derivatives, extracellular vesicles (EVs) are included [7].

EVs participate in several biological processes both in physiological and pathological human diseases. Specifically in cancer, EVs play an essential role in different oncogenic processes, including cell-to-cell communication, the shaping of the tumour microenvironment (TME), epithelial–mesenchymal transition, and pre-metastatic niche formation [8][9]. The EV cargo frequently differs between tumour- and healthy-derived EVs. EVs can be recovered from several body fluids, and in particular from blood, urine, Broncho-Alveolar Lavage Fluid (BALF), ascites, and cerebrospinal fluid (CSF).

Given these peculiarities, EVs hold great potential as cancer biomarkers and diagnostics. In this review, evidence on EVs, as principal and/or ancillary diagnostic biomarkers, in the most clinically relevant neoplastic diseases involving the CNS, head and neck, lung, and the gastrointestinal tract, are discussed.

2. Head and Neck Cancers

Saliva is considered an easily available and noninvasive source for early biomarker detection in high-risk oral cancer (OC) patients [10]. On the contrary, the whole saliva is a more appropriate source for biomarker detection of

different OCs, as malignant EVs can be recovered from the whole saliva simply by bathing the oral cavity [11]. [10] have demonstrated that the oral fluid from OC patients contains EVs that differ morphologically and molecularly from those obtained by oral fluids of OC-free individuals. [12] analyzed 21 patients with oral squamous cell carcinoma (OSCC) and 11 controls, by isolating EVs from saliva.

In a different study, salivary EVs enriched in miR-24-3p were selected among three upregulated mRNAs in OSCC. [13] have reported that miRNA-27b is significantly upregulated in EVs recovered from the saliva of OSCC patients. The authors have shown its high sensitivity and specificity in detecting OSCC compared to other miRNAs, and its efficacy in distinguishing OSCC patients in remission and individuals with oral lichen planus. [14] reported a significant increase in salivary EV-miR-21 and miR-184 and a significant decrease of EV-miR-145 in OSCC patients when compared to healthy subjects and patients with recurrent aphthous stomatitis.

[15] have demonstrated the overexpression of cyclophilin A (CYPA) in sera, tissues, and circulating EVs of patients suffering from nasopharyngeal cancers (NPCs). In particular, the ROC curves exploited to analyse the diagnostic values of the whole sera and the EV-CYPA content demonstrated that the AUCs correspond to 0.631 (CYPA detected from the whole sera; $p = 0.042$) and 0.844 (EV-CYPA; $p < 0.0001$), indicating that EV-CYPA enrichment has a higher diagnostic significance than the serum CYPA content. They demonstrated that a combination of EV-CYPA content and EBV-VCA-IgA increases the diagnostic accuracy, particularly in patients negative for EBV-VCA- The authors detected baseline circulating HPV-DNA in cf-DNA from 21 out of 23 HPV-OPCSCC cases (91% sensitivity), while the HPV-DNA in circulating EVs was only detectable in 8 out of 19 patients (42% sensitivity).

[16] compared the EV-miR-21 and HOTAIR content in the serum of patients suffering from laryngeal squamous cell carcinoma (LSCC) and polyps of the vocal cords. Overall, the combo was able to differentiate malignant neoplasms from benign laryngeal disease with 94.2 and 73.5% of sensitivity and specificity, respectively. [17] focused on the identification of markers allowing a differential diagnosis among lung squamous cell carcinoma (LSQCC), solitary metastatic lung tumour (MSQCC) and head and neck squamous cell carcinoma (HNSQCC). The validation dataset with formalin-fixed paraffin-embedded (FFPE) from MSQCC and LSQCC demonstrated that miR-10a, miR-28, miR-141, and miR-3120 enriched in circulating EVs were significantly higher in the LSQCCs than the MSQCCs and HNSQCC, while the expression of the same miRNAs in circulating EVs from the LSQCC patients was significantly higher than those from the MSQCC patients.

3. Lung Cancers

However, in clinical practice up to two-thirds of lung cancers are diagnosed at stage III (locally advanced) or VI (metastatic cancers) which constricts the therapeutic success [2]. However, when the EV-associated miRNAs were considered, only miR-19-3p, miR-21-5p, and miR-221-3p were found to be significantly upregulated in lung cancer patients. In lung adenocarcinoma patients, all miRNAs were upregulated, while in lung granulomas a slight downregulation of miR-139-5p, miR-30a-3p, and miR-378a was detected. The downregulated miR-382-3p in lung adenocarcinoma patients compared with healthy controls displayed an 85.7% sensitivity and a 95.8% specificity [18].

Only a few studies tried to identify EV-associated lipids as cancer biomarkers. Among them, Fan et al. [19] performed the lipid profiles of EVs obtained from normal and NSCLC subjects and applied two multivariate statistical methods, the Random Forest (RF) and the Least Absolute Shrinkage and Selection Operator (LASSO), to select 23 lipids. The authors were able to discriminate between early- and late-stage cancers and controls (AUC corresponding to 0.85 and 0.88 for RF and 0.79 and 0.77 for LASSO, respectively).

PE is a relatively common clinical presentation of lung cancer [20]. EVs are present in the PE and may serve for the differential diagnosis of lung cancers. [21] have compared EV-associated miRNAs in the PE of patients suffering from pneumonia, pulmonary tuberculosis, and lung cancers. Therefore, the combination of radiological screening (low dose CT) and EV-based strategies would represent the future challenge to reduce the rate of false positive results and allow for greater accuracy in lung cancer screening.

4. Cancers of the Gastrointestinal Tract

It embodies two distinct major histopathologic types: squamous cell carcinoma (ESCC) and adenocarcinoma (EAC) The epidemiology of oesophageal carcinoma widely differs worldwide. Regarding the pathology of the oesophageal carcinoma, EAC represents the most common histological subtype. Non-invasive diagnostic and screening protocols, offered to high-risk individuals (e.g., familial history of oesophageal carcinoma, smokers, heavy alcohol drinkers), might therefore allow an early therapeutic approach and a better prognosis.

[22] highlighted how circulating EVs from ESCC patients are stable enough and are significantly higher in serum from ESCC patients compared with controls. However, no significant association among EV number, gender, age, tumour size, lymph node invasion, metastasis, tumour grade, and UICC stage was detected. Takeshita and colleagues [23], isolated EVs from venous blood samples obtained by 101 ESCC patients and 46 healthy controls to perform miRNA profiling. The authors also demonstrated that the expression of miR-1246 correlates with tumour stage, lymph node status, metastatic burden, and with a 2-year OS.

Their expression in EVs demonstrated that miR-223-3p and miR-584 were dysregulated consistent with their plasma level. On the contrary, miR-20b and miR-486-5p were significantly downregulated in EVs from ESCC patients compared to healthy individuals. The authors demonstrated that, from among these miRNAs, only miR-486-5p was deregulated in the plasma, EVs, and tumour tissues. Interestingly, when the diagnostic potential of free plasma miRNAs and EV-miRNAs were compared, EV-miR-223-3p content displayed a higher diagnostic accuracy than plasma miR-223-3p (AUC corresponding to 854).

Stathmin-1 is a microtubule-destabilizing cytosolic phosphoprotein, which is post-transcriptionally regulated by miR-34a, miR-223, and miR-193b, and plays a central role in tumour cell proliferation and migration. Stathmin-1 is associated with metastatic disease and poor prognosis in osteosarcoma, prostate cancer, head and neck squamous cell carcinoma, hepatocellular carcinoma, colorectal cancer, gallbladder carcinoma, and non-small-cell lung cancer [24][25][26][27]. Likewise, in ESCC, stathmin-1 was linked to tumour invasiveness and is proposed as a

predictor of poor prognosis. The authors concluded that stathmin-1 is an outstanding diagnostic and predictive marker for squamous cell carcinoma, particularly for ESCC.

In a different study, a chimeric EV-mRNA, the seG-NchiRNA, was proposed as a biomarker to discriminate the early and advanced ESCC stage, as well as for the postoperative surveillance, therapeutic response, and tumour recurrence. The authors confirmed that the intracellular G-NchiRNA in ESCC cells closely correlated with the salivary EV-G-NchiRNA content. Moreover, it was reported that the G-NchiRNA increased with tumour growth, and that the amount of G-NchiRNA in tumour lysates significantly correlated with its content in salivary and serum EVs [28] (Table 6).

Therefore, the downregulation of miR-23b has been considered a potential marker for diagnostic purpose and for monitoring tumour relapse. Zhang and colleagues [29] analysed EV-miRNA content in patients with GC at different stages: primary Gastric Cancer (pCG), GC with lymph node metastasis (GCln), GC with ovarian metastases (GCo), and GC with liver metastases (GCl). [30] have proposed the increased EV-HOTTIP content as a potential GC biomarker by analysing its expression in the sera of 246 patients (126 GC samples and 120 controls). Although endoscopy is required for a conclusive diagnosis, it is conceivable to assume that the combination of standard biomarkers and EV-specific cargoes may be useful to better select patients requiring invasive diagnostic procedures.

The mortality in CRC patients has been estimated to be approximately 609,000. [31] who first described 13 dysregulated miRNAs in Ep-Cam positive EVs purified from peripheral blood samples of CRC patients and controls. Additionally, 11 miRNAs (miR-23a, miR-92a, miR-221, miR-301a, miR-31, miR-143, miR-142, miR-223, miR-18a, miR-135b, and miR-18b) were found to be dysregulated in serum EVs from CRC patients by Karimi et al. A lower diagnostic accuracy, but one worth mentioning, was reported for the enrichment of miR-486-5p [32] and miR-6803-5p [33] in EVs from CRC patients (Table 5).

[34] also identified the heat shock protein 60 (Hsp60) in EVs recovered from CRC patients before and after the removal of primary tumours. Glypican-1 (GPC1)-positive circulating EVs from CRC patients have been proposed for diagnostic purposes [35]. The percentage of GPC1 (+) EVs was found to be markedly increased and normalized after surgery. EV-CPNE3 content showed a better diagnostic power than CEA and, the combination of EV-CPNE3 and CEA was found to be superior to EV-CPNE3 or CEA to identify cancer patients (Table 6).

Lydic et al. [36] performed a lipidome profiling of EVs secreted by the colorectal cancer cell line LIM1215. However, no specific diagnostic marker was identified.

Pancreatic cancer (PC) is the seventh leading cause of global cancer deaths in industrialized countries. PC is mainly divided into two subtypes: Pancreatic Adenocarcinoma and Pancreatic Neuro Endocrine Tumours (NETs). The pancreas adenocarcinoma is more aggressive than the NETs, with an OS corresponding to 24% 1 year after diagnosis, and 9% at 5 years. More specifically, the timing of the diagnosis, as 80–90% of patients present with an unresettable pancreatic tumour at diagnosis [5][37].

The analysis of circulating EV-miRNA content in 40 patients (29 with PC and 11 healthy controls or chronic pancreatitis) allowed Lai et. In a different study, EV-miR-191, miR-21, and miR-451a were found significantly upregulated in patients with PC and (SNORA14B, SNORA18, SNORA25, SNORA74A, and SNORD22) were analysed in circulating EVs from patients with PC and controls. EV level in 24 PC patients and 32 patients with non-neoplastic pancreas disease or healthy controls.

Hepatocellular carcinoma (HCC) is a primary liver cancer that usually develops in the context of chronic liver diseases [38]. In addition, chronic hepatitis, HBV or HCV infection, alcohol consumption, and liver cirrhosis are considered to be the most relevant associated risk factors. The circulating EV level was evaluated in patients with HCC (n = 55), liver cirrhosis (n = 40), and healthy subjects (n = 21). Moreover, the authors proposed a diagnostic algorithm for patients at high risk for liver cancer also including EV characterization.

Chapuy-Regaud et al. [39] performed a lipidomic analysis on EVs released from uninfected and HEV-infected cells and showed a differential amount of free cholesterol, ceramides, phosphatidylserine, sphingomyelin, and phosphoinositides. Similarly, Haraszti et al. [40] analysed the lipid composition in EVs from Huh7 hepatocellular carcinoma cells and human MSCs and did not find significant differences.

Overall, these results strengthen the notion that EV-related markers may strongly influence the diagnosis of liver disease and early liver cancer compared to conventional biomarkers such as AFP.

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