Clinica Chimica Acta 490 (2019) 186–193

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

The potential of exosomes derived from colorectal cancer as a biomarker

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ARTICLE INFO

Keywords:
Colorectal cancer
Exosomes
Exosomal tumor markers
CK19
TAG72
CA125

ABSTRACT

Colorectal cancer (CRC) is one of the most frequent causes of cancer death. The diagnosis and treatment for metastatic CRC and patients with drug resistance remains poor. Cancer cells characteristically produce and release exosomes, which act as a new signal pattern in the occurrence and development of tumors. Circulating exosomes constitute promising biomarkers for early diagnosis of cancer patients. However, the potential of exosomes for clinical biomarker is hampered by their variousness and multi-sources. To this aim, we set out to solve the problem of discrimination and classification of exosomes. The cell culture supernatants of CRC cells, renal cell carcinoma cells and breast cancer cells were used for isolating exosomes. Then characteristic exosomal tumor markers were explored by Roche Cobas E601 fully automated electrochemiluminescence immunoassay systems, western blot and flow cytometry analysis. The results showed that CK19 was commonly expressed in exosomes derived from colorectal cells, TAG72 was mainly expressed in exosomes of 5-FU-resistant CRC cells and CA125 in those of high metastatic CRC cells. In addition, tumor interstitial fluid derived exosomes and patients' plasma derived exosomes showed different levels of CK19, TAG72 and CA125. Collectively, these data indicated that exosomes derived CK19 is correlated with colorectal tissue. TAG72-rich exosomes indicate that CRC patients might be resistant to 5-Fluorouraci. CA125-rich exosomes might be as metastatic CRC markers. These provide a good prospect for cell exosomes as novel, non-invasive clinical tool for diagnosing CRC and predicting its progression.

1. Introduction

The molecular diagnosis and surveillance of diseases is supported by many biomarkers [1]. Body fluids are the most attractive biomarker sources for large-scale noninvasive clinical detection. But the biofluid-derived soluble biomarkers present an enormous challenge for diagnostic accuracy with sample complexity [2]. For cancer screening, it should be kept firmly in mind that plasma-derived biomarkers are not linked to specific development of tumors [3]. But for lots of cancers, if detected at an early stage, most have the access to be curable by some conventional therapies, such as surgical treatment, chemotherapy and immunotherapy [4]. So the promising biomarkers for early diagnosis of cancers are really needed.

Building on the fact that cancer cells can characteristically produce and release extracellular vesicles (EVs), particularly of nanosize vesicles called exosomes, which carry a series of cargoes including proteins and nucleic acids [5], we set out to seek the more available and efficient biomarkers in this noninvasive field. Exosomes are first described by Tram and co-authors in 1981 [6], since then medical and clinical interest has increased rapidly. Exosomes are round or cup shaped with size between 40 and 150 nm and density between 1.13 and 1.19 g/ml [7,8]. Of interest, exosomal biomarkers and molecule information remain relatively stable owing to be protected from degradation and external impact [9].

Constantly emerging data elucidate the relationship between exosome production and tumorigenesis. The key role of the interaction

Abbreviations: CRC, colorectal cancer; CK19, cytokeratin 19; TAG72, tumor-associated glycoprotein 72; CA125, carbohydrate antigen 125; EVs, extracellular vesicles; FBS, fetal bovine serum

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https://doi.org/10.1016/j.cca.2018.09.007
Received 7 August 2018; Received in revised form 28 August 2018; Accepted 3 September 2018
Available online 06 September 2018
0009-8981/

between cancer cells and tumor microenviroment has been pointed and exosomes act as a key mechanism in the occurrence and development of tumors [10]. Evidence has also shown that the level of plasmatic human EVs increases along with the tumor growth, so increased exosomal markers could be tested in the body fluids,thus we can describe the circulating exosomes as "circulating tumor mass" in cancer patients [11,12]. Notwithstanding, the potential of exosomes for clinical biomarker test is hampered by their variousness. There are diversiform exosomes derived from both normal cells and tumor cells, hepatic cells and renal cells, and so on [13], we cannot obtain precisely useful information if we have no way to classify exosomes.

In this study, we searched for exosomal tumor markers and differentially expressed proteins were screened. Our results revealed that exosomal markers had crucial roles in tumor microenvironment, which subsequently identified CRC metastasis and chemoresistance.

2. Materials and methods

2.1. Cell culture

CRC cell lines LoVo, HCT-8 and 5FU resistant CRC cell line (HCT-8/5FU), renal cell carcinoma cell line ACHN, breast cancer cell line MCF-7 and the corresponding normal cell lines FHC, HEK293T and MCF-10A were used. HCT-8, HCT-8/5FU, FHC, HEK293T and MCF-10A cells were cultured in RPMI supplemented with 10% exosome-depleted FBS and 1% penicillin-streptomycin. LoVo, MCF-7, and ACHN cells were cultured in DMEM supplemented with 10% exosome-depleted FBS and 1% penicillin-streptomycin. In addition, the exosome-depleted FBS was prepared as follows, FBS was filtered with 0.22 μm filter, then centrifuged at 120,000*g* for 5 h to eliminate the exosomes, then filtered again with 0.10 μm filter.

2.2. Exosomes isolation from cell culture supernatants

Exosomes were isolated by differential centrifugation processes [14]. Supernatants from $1 * 10^7$ cells were harvested after 2 days at 80–85% confluent. After centrifugation of cells at 1000*g* for 20 min, the supernatants were then centrifuged at 12,000*g* for 30 min. The supernatants were filtered using 0.22 μm filter and centrifuged at 120,000*g* for 2 h at 4 °C using a Hitachi CP100WX Ultracentrifuge (*Hitachi* Limited, Tokyo, Japan) to pellet exosomes. The exosomes were resuspended in PBS (100 μl) for subsequent experimental analysis.

2.3. Nude Mouse Xenograft and exosomes enrichment from tumor interstitial fluid

Four-week-old male BALB/c athymic nude mices were purchased from the Animal Facility of Dalian Medical University. The animal experiments were approved by the Committee on the Ethics of Animal Experiments of the Dalian Medical University, China. Briefly, the cells were harvested and $2 * 10^6$ cells were injected subcutaneously into the flanks of the nude mouse. When the mice appeared obvious tumors, the mice were sacrificed and tumors were harvested. Fresh tumor tissues were used for preparing tumor interstitial fluid. In order to avoid tissues damage and minimizing cell lysis, tumor interstitial fluid was dependent on gentle manipulation [15]. 0.5 g fresh tumor tissues were cut into small pieces (about 2–3 mm^3), washed in 5 ml of phosphate buffered saline (PBS), and placed into a 10-ml EP tube containing 1 ml PBS. Samples were incubated for 90 min at 37 °C in a humidified CO_2 incubator. Thereafter, the samples were centrifuged at 500*g* for 2 min. Samples were further centrifuged at 3000*g* for 20 min at 4 °C. The supernatant was centrifuged at 12,000*g* for 30 min and then filtered using 0.22 μm filter. Final supernatant was centrifuged at 120,000*g* for 2 h at 4 °C using a Hitachi CP100WX Ultracentrifuge to pellet exosomes. All the final exosome pellets were resuspended in PBS (100 μl) for subsequent experimental analysis.

2.4. Human plasma samples preparation and exosomes isolation

Human plasma samples were collected from patients with CRC diagnosed at First Affiliated Hospital of Dalian Medical University (Dalian, China) ($n = 40$). The plasma samples were separated into two groups according to the clinical conditions: 1. Advanced metastasis group (AM) ($n = 18$). 2. Early non metastasis group (ENM) ($n = 22$); Colorectal cancer patients who had poor response to 5-FU chemotherapy or underwent the replacement of chemotherapeutics were also selected according to retrospective clinical state, recording as drug resistant group (DR) ($n = 18$). Plasma samples of health checkup were treated as normal control group (NC) ($n = 35$). The plasma of each group were aggregated into a total pool and aggregate four pool plasma samples were used to isolate exosomes with ExoQuick™ (System Biosciences). The study has been examined and certified by the Ethics Committee of the First Affiliated Hospital of Dalian Medical University (YJ-KY-FB-2016-16), and informed consent was obtained from all participants included in the study, in agreement with institutional guidelines.

2.5. Electron microscopy and exosome size and density measurements

Exosomes suspensions were placed onto 200 mesh carbon-coated grids at an optimal concentration and allowed to be absorbed to the velamen for 3 min. Then grids were allowed to dry for 1 min and stained for contrast using 3% phosphotungstic acid. The samples were viewed with a JEM-2000EX transmission electron microscope (JEOL, Japan) and images were taken in a suitable proportion. The size and density of exosomes were measured by Zetasizer Nano (Malvern, England). Briefly, exosome-enriched pellets were resuspended in 1 ml of 0.1 μm triple-filtered sterile PBS. Collected data were analyzed with Zetasizer Nano software, which provided size distribution report by intensity.

2.6. Automated electrochemiluminescence immunoassay system for exosomal tumor markers searching

To search the exosomal tumor markers, exosomes isolated from CRC cells, renal cell carcinoma cells, breast cancer cells and corresponding normal cells were homogenized in RIPA lysis buffer. Then equal amounts of exosomal protein lysates were detected to seek the valuable markers using Roche Cobas E601 fully automated electrochemiluminescence immunoassay system. Exosomes derived from different tumor cells were chosen and normal cell-secreted exosomes as control, which made the result comparative and practical. Differential protein or other markers were selected and confirmed.

2.7. Western blot analysis

To deduce the protein levels in exosomes, exosomal protein lysates were normalized using Bicinchoninic Acid (BCA) protein assay kit (Thermo Fisher Scientific, Massachusetts, USA). 20 μg protein lysates were loaded on 12% polyacrylamide gels and transferred to a polyvinylidene difluoride membrane. The membranes were blocked with 5% skimmed milk and incubated with CK19 antibody, TAG72 antibody, HSP70 antibody and CD63 antibody (1/1000 dilution; Abcam, Cambridge, UK) and CA125 Monoclonal antibody (1/1000 dilution; Thermo Fisher Scientific, Massachusetts, USA) at 4 °C overnight, followed by incubation with anti-rabbit IgG (1/5000 diluted; GE Healthcare UK Ltd., Little Chalf-ont, UK) at 37 °C for 2 h. All bands were detected using ECL Western blot kit (Amersham Biosciences, UK).

2.8. Flow cytometry analysis of exosomes

Exosomes from cell supernatants, tumor interstitial fluid and patient's plasma samples were isolated and resuspended in PBS. The beads

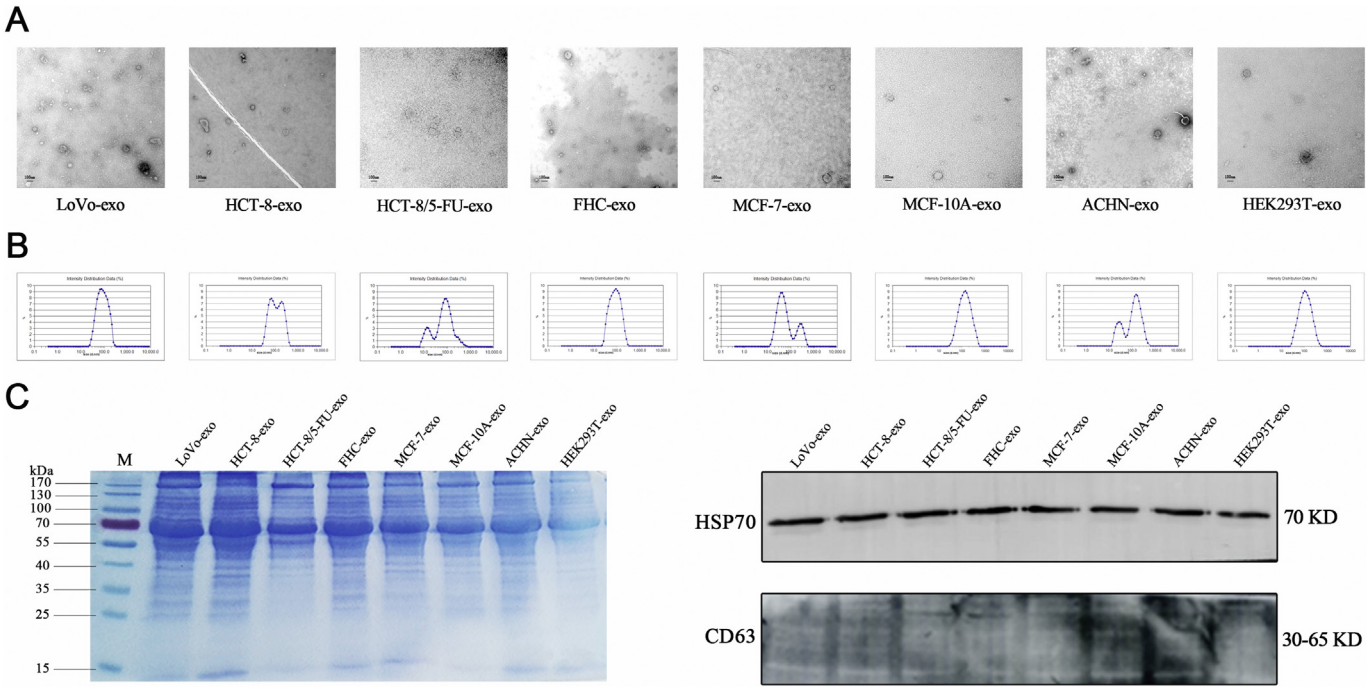

Fig. 1. Identification of exosomes derived from cell culture supernatants (A) Transmission electron microscopy showing 40–150 nm in diameter, round or cup-shaped exosomes, derived from CRC cell lines LoVo (LoVo-exo), HCT-8 (HCT-8-exo) and 5-FU-resistant CRC cell line (HCT-8/5FU-exo) and normal colon cell line FHC (FHC-exo); Breast cancer cell line MCF-7 (MCF-7-exo) and normal immortalized breast epithelial cell line MCF-10A (MCF-10A-exo); Renal cell carcinoma cell line ACHN (ACHN-exo) and Human embryonic kidney cell line HEK293T(HEK293T-exo). **(B)** Size distribution of exosomes derived from eight cell lines was measured by Zetasizer Nano. **(C)** Western blot analysis of exosomes from eight cell lines. The total protein concentration was checked with coomassie brilliant blue staining (left panel), then CD63 and HSP70 was blotted (right panel).

with anti-HSP70 antibodies attached were prepared as follows, latex beads (10 μl) were added to 40 μl PBS with 3 μl anti-HSP70 and the mixture were allowed to mix using bench-top rotator overnight at 4 °C. Then the mixture was spun down at 12,000 r.p.m. for 2 min and the supernatant was aspirated. The pellet was washed three times with PBS and the beads with anti-HSP70 antibodies attached were resuspended in 200 μl PBS. Then 100ul exosome solutions were added and mixture was allowed to mix using bench-top rotator for 10 h. The solution was then centrifuged at 14000 *g* for 2 min. The pellet was then resuspended in 100 μl of 10% BSA in PBS, and mixed for 45 min. The mixture was spun down at 12,000 r.p.m. for 2 min and the supernatant aspirated. The beads with exosomes attached (pellet) were then resuspended in 100 μl of 2% BSA in PBS, and spilt equally into different tubes, staining for CD63, CK19, TAG72 and CA125, secondary antibody only for control. The exosomes bound to beads were then incubated with 3 μl primary antibody in 20 μl volume and mixed for 30 min. The mixture was then spun down at 12,000 r.p.m. for 2 min, the supernatant aspirated and pellet resuspended in 50 μl of 2% BSA in PBS. Secondary antibodies labeled with FITC were then added and the samples were mixed for 60 min. The mixture was spun down at 12,000 r.p.m. for 2 min and the supernatant was aspirated. The pellet was washed three times with 2% BSA in PBS and the exosomes bound to beads were resuspended in 200 μl of 2% BSA in PBS. CD63 and other specific antigens detection on the latex bead-exosome combinant were analyzed using ACEA NovoCyte flow cytometry.

2.9. Rapid detection methods for exosomes using the Enzyme-linked immunosorbent assay (ELISA)

Plastic 96-well plates were coated with 3 μg/μl rabbit anti-HSP70 antibody and incubated overnight at 4 °C and blocked with 0.5% BSA. PBS with 0.05% Tween-20 (PBST) was used as washing buffer. Then exosomes suspension isolated from plasma samples of human patients were added to plate wells (final volume of 100 μl) and incubated for 3 h. Samples were washed three times with PBS and then incubated with primary antibodies (anti-CK19, anti-TAG72, or anti-CA125) diluted in sample buffer (0.5% BSA PBS) for 2 h at 4 °C. After extensive washing, the plate was incubated with HRP-conjugated Secondary Antibody (BioRad, California). Upon addition of the BM Blue POD Substrate (Roche Applied Science, Milan) optical densities were recorded at 450 nm.

2.10. Immunofluorescence and immunohistochemistry

CRC cells were confirmed for specific molecular markers using immunofluorescence, cells were washed with PBS, fixed with 3.7% formaldehyde, and permeabilized with PBS containing 0.1% Triton X-100 (Sigma). After being incubated with primary antibody overnight at 4 °C, cells were incubated with fluorescently-labeled secondary antibody. DAPI was used for nuclear staining and pictures were taken with fluorescence microscope. Tumors retrieved from mices were immediately immersed in 4% buffered formaldehyde, washed, dehydrated and finally embedded in paraffin. Tumor slices were deparaffinized and incubated with the primary antibodies and then the secondary streptavidin-horseradish peroxidase-conjugated antibody. Staining was performed at room temperature, visualized in 3,3′-diaminobenzidine, counterstained with hematoxylin, dehydrated and mounted for visualization.

2.11. Statistical analysis

Comparisons of means of ≥3 groups were performed by analysis of variance (ANOVA), followed by Tukey's post-hoc test. The data were represented as the mean ± standard deviation (± SD) from three independent experiments. The p values of < 0.05 considered that the difference was significant compared with control.

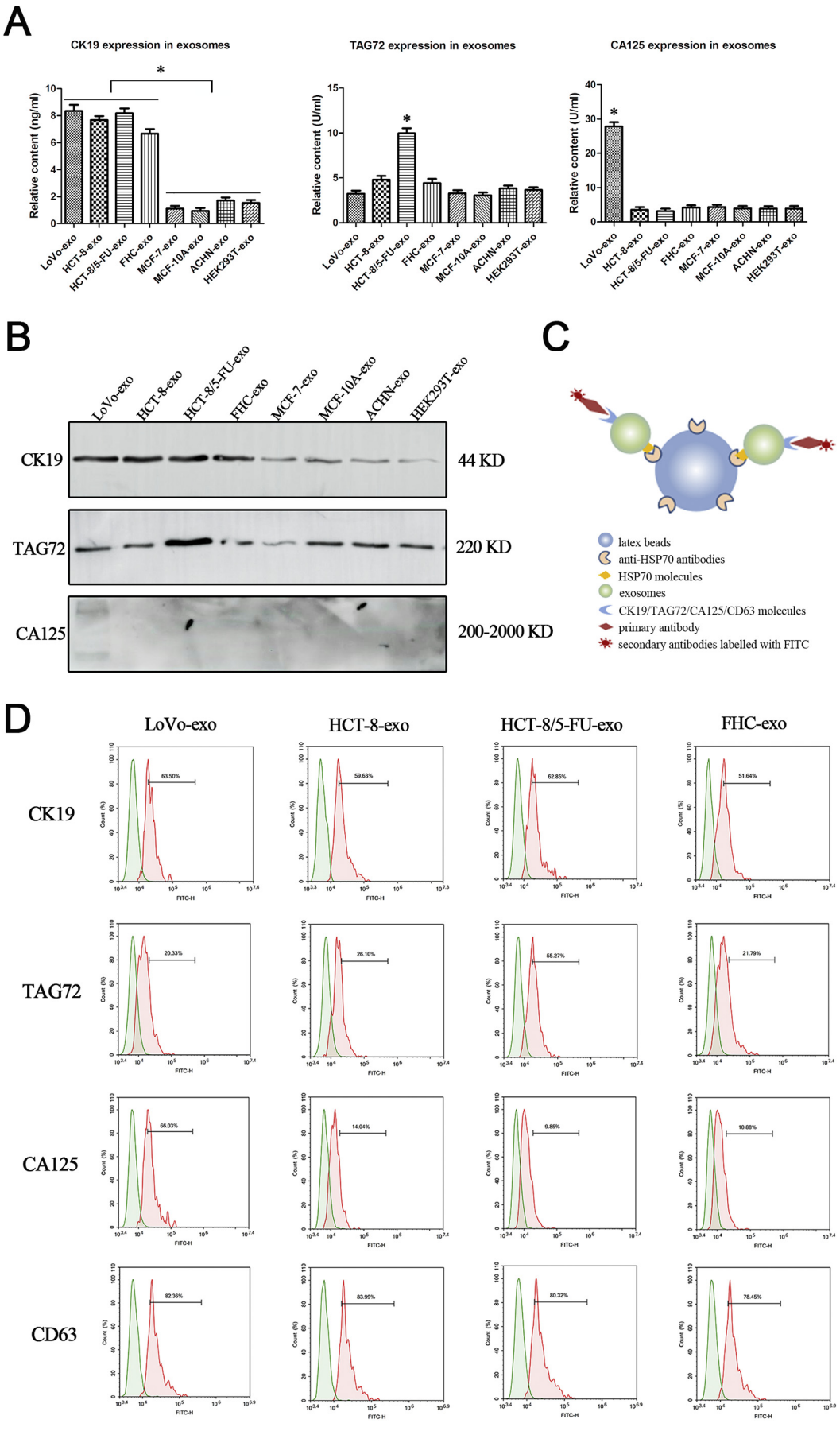

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Fig. 2. Identification of exosomal tumor markers (A) The screening results of exosomal tumor markers by Roche Cobas E601 fully automated electrochemiluminescence immunoassay system indicated that CK19 was higher expressed in exosomes derived from the cell of the colorectal tissue than that of other two tissue cells, and TAG72 was mainly expressed in exosomes derived from 5-FU-resistant CRC cells and CA125 in that of high metastatic CRC cells. **(B)** Western blot analysis of CK19, TAG72 and CA125 for confirming the exosomal tumor markers. **(C)** Schematic representation of the latex bead-exosome combinant for flow cytometry analyses. **(D)** Flow cytometry analyses of exosomal tumor markers CK19, TAG-72 and CA125, CD63 used as exosomal marker control. Presented data are representative of three independent experiments and reported as means ± SD, $*P < .05$.

3. Results

3.1. Identification of exosomes derived from cell culture supernatants

The presence of exosomes was confirmed by transmission electron microscopy and images indicated that exosomes derived from CRC cells, renal cell carcinoma cells, breast cancer cells and corresponding normal cells were 40–150 nm in diameter, round or cup-shaped and enclosed by a lipid bilayer (Fig. 1A). The size and density of exosomes were measured by Zetasizer Nano, the results confirmed that nanoscale vesicles were isolated successfully (Fig. 1B). Exosomes were also characterized for typical exosomal markers CD63 and HSP70 by western blot (Fig. 1C). These results suggested that the exosomes were released from both normal cells and tumor cells, and exosomes derived from different tissue cells could not be differentiated only on the basis of diameter and morphology. So there was multifarious variety of exosomes existing in our body fluids.

3.2. Identification of exosomal tumor markers

The screening results of exosomal tumor markers indicated that CK19 was generally higher expressed in exosomes derived from CRC cell lines than that of two other tumor cell lines. The normal human colon *cell line* (FHC) secretory exosomes also expressed a certain amount of CK19, suggesting that CK19 could be treated as a biomarker for exosomes of the colorectal tissue. TAG72 and CA125 were also higher expressed in CRC cell secretory exosomes. TAG72 was mainly expressed in exosomes derived from 5-FU-resistant CRC cells, while CA125 was overexpressed in metastatic CRC cell lines (Fig. 2A). Western blot (Fig. 2B) and flow cytometry (Fig. 2D) had further verified the screening results. The schematic representation of the latex bead-exosome combinant used for flow cytometry analysis had been shown (Fig. 2C). Differential expression of CK19, TAG72 and CA125 in CRC exosomes might be as special markers for CRC diagnosis. The three molecular markers were also confirmed to exist in the CRC cells, having similar distribution trends with exosomal markers (Fig. S1A, B and C).

3.3. Exosomes enrichment and identification from tumor interstitial fluid

Highly metastatic cells (LoVo), low metastatic cells (HCT-8) and 5-FU-resistant CRC cells (HCT-8/5FU) were used for tumor xenografts, and normal colorectal tissue as control (Fig. 3A). Tumor interstitial fluid was collected and exosomes were isolated smoothly. Transmission electron microscopy images revealed that exosomes derived from tumor interstitial fluid showed a state of obvious enrichment (Fig. 3B). The typical exosomal markers CD63 and HSP70 were analyzed. CK19 was commonly expressed in exosomes derived from four kinds of interstitial fluids. TAG72 was mainly expressed in exosomes of HCT-8/5FU tumor interstitial fluid and CA125 in LoVo tumor interstitial fluid (Fig. 3C). Flow cytometry analysis had also confirmed these results (Fig. 3D). Exosomal tumor markers of interstitial fluids were consistent with cell supernatants, suggesting that distinct cells could secrete differential exosome population. In addition, immunohistochemistry of CK19, TAG72 and CA125 were detected in the tumors retrieved from mice, also with the similar distribution trends with exosomal markers (Fig. S1D).

3.4. Exosomes isolation and detection from plasma samples of CRC patients

The plasma samples of CRC patients were divided into three groups as the rules described above: 1. Advanced metastasis group (AM); 2. Early non metastasis group (ENM); 3. Drug resistant group (DR); Exosomes were isolated from equal amounts of plasma samples of three patient groups and normal control group (NC) and confirmed by Transmission electron microscopy (Fig. 4A) and western blot (Fig. 4B). The exosomal suspensions derived from patients were tested for exosomal tumor markers. Flow cytometry (Fig. 4C) and ELISA analysis (Fig. 4D) further confirmed the results. Overall, these findings suggested that exosomal CK19 was correlated with colorectal tissue. TAG72-rich exosomes indicated that CRC patients might be resistant to 5-Fluorouraci. CA125-rich exosomes might be as metastatic CRC markers.

4. Discussion

CRC is the third commonly diagnosed malignancy and one of the most frequent causes of cancer death worldwide [16,17]. In recent years, surgical resection combined with chemotherapy for non-metastatic CRC, has developed with rapid progression. However, the diagnosis and treatment for patients with metastatic CRC remains poor [18]. The current approach to treating metastatic CRC favors the combination cytotoxic therapy, and 5-FU is a classical and commonly used clinical chemotherapy [19]. The clinical application of 5-FU is limited by drug resistance, with only a 10–30% successful therapy rate [20]. Therefore, the promising biomarkers for early diagnosis of CRC are really needed.

Exosomes are the membrane-bounded compartments that transport proteins, lipids and nucleic acids [21] between different cells, which could travel large distances in body fluids or the bloodstream. This information-transfer paradigm has attracted considerable attention in cancer research, because some extracellular vesicles carry cancer-causing genes called oncogenes, or oncogenic proteins that promote cancer progression [22]. Exosomes participate in tumorigenesis and metastasis [23]. Cancer cells known to preferentially home to the lung, liver, brain or bone could produce exosomes, which selectively interact with the same organ for tumor metastasis. Additionally, exosomes function as mediators of drug resistance. Drug resistance information is exchanged via transport of exosomal proteins such as multi-drug resistant protein P-gp and cytotoxic drugs cisplatin [24–26]. As exosomes enable the exchange of information among tumor cells, the specific nucleic acids and proteins they carry, have attracted the application in the early diagnosis and treatment of tumors. Being as clinical tumor markers, exosomes widely existence in all body fluids, including saliva, blood, urine, cerebrospinal fluid, pleural effusion and ascites [27]. Tumor derived exosomes are only one subgroup that really represents the characteristics of the tumor cells and carry valuable information for diseases. So identification of exosomal tumor markers is pretty necessary when exosomes could be used as cancer markers for humoral examination.

The identification of exosome subsets has attracted attention of researchers. Exosomal tumor biomarkers are used in cancer patients, such as MART-1 for melanoma [28], PSA for prostate cancer [4] and Heat shock protein 60 (Hsp60) as a good candidate biomarkers for large bowel carcinoma [29]. Here, to search the exosomal tumor markers from CRC cells, renal cell carcinoma cells and breast cancer cells,

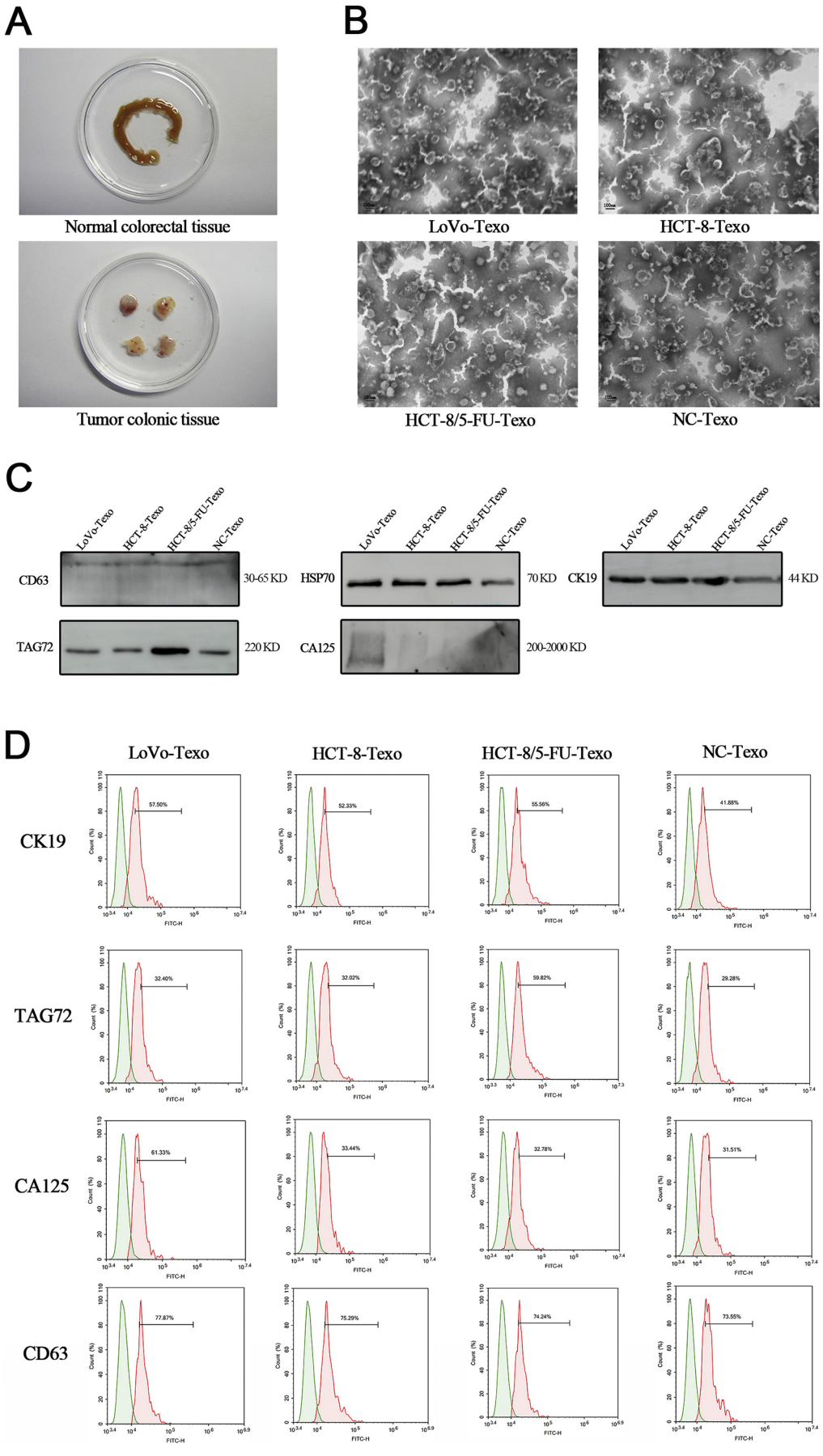

Fig. 3. Exosomes enrichment and identification from tumor interstitial fluid (A) CRC cells were successfully used to establish nude mouse xenograft, normal colorectal tissue used as control for preparation of interstitial fluid. **(B)** Transmission electron microscopy showing exosomes enriched from LoVo tumor interstitial fluid (LoVo-Texo), HCT-8 tumor interstitial fluid (HCT-8-Texo), HCT-8/5FU tumor interstitial fluid (HCT-8/5FU-Texo) and normal colorectal tissue interstitial fluid (NC-Texo). **(C)** Western blot analysis of CD63, HSP70 and exosomal tumor markers CK19, TAG72 and CA125 in exosomes enriched from tumor interstitial fluid as mentioned above. **(D)** Flow cytometry analysis of exosomal tumor markers CK19, TAG72 and CA125 in tumor interstitial fluid-derived exosomes, CD63 used as exosomal marker control.

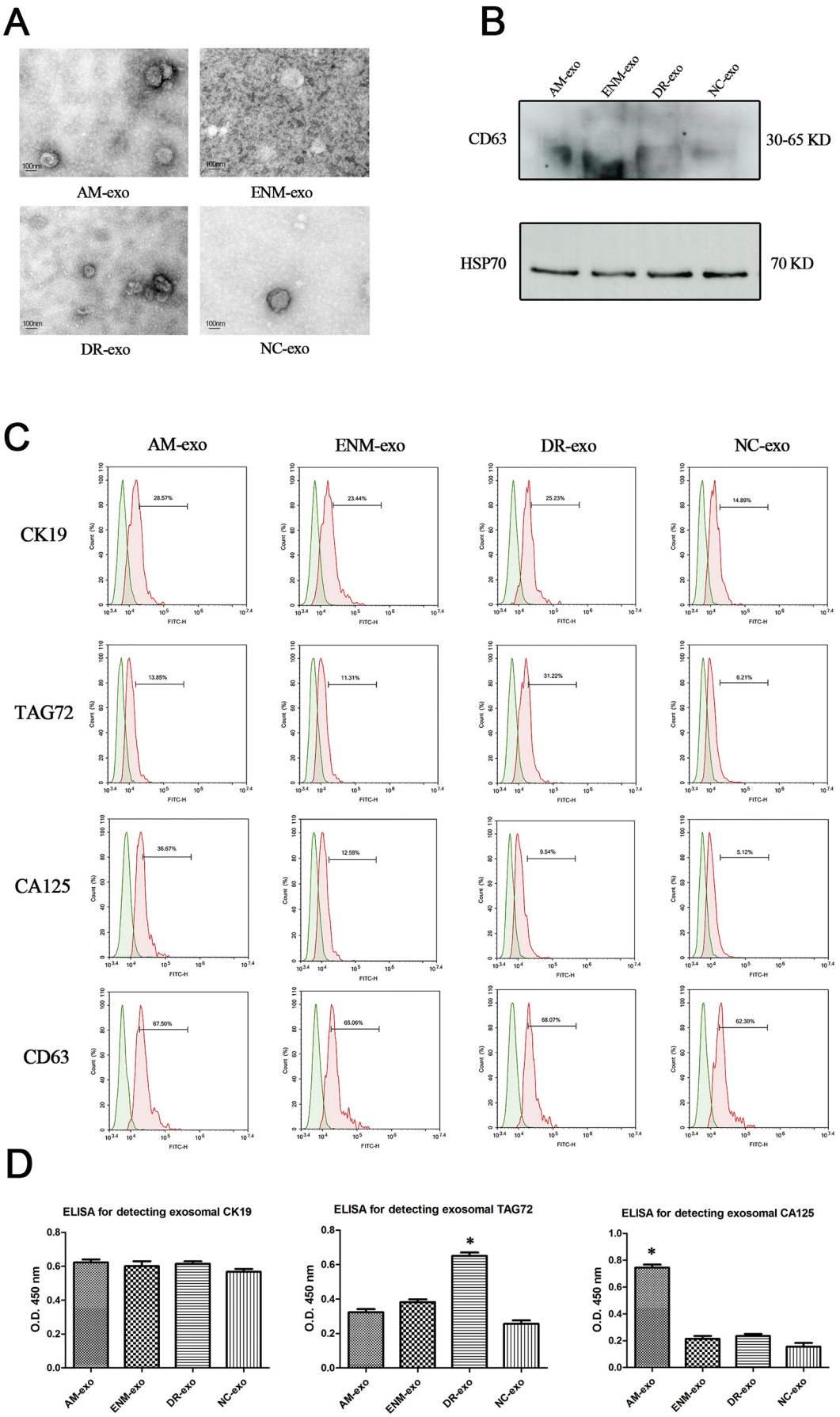

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Fig. 4. Exosomes isolation and detection from plasma samples of CRC patients (A) The plasma samples of CRC patients were collected and divided into three groups according to the clinical condition, plasma samples of health checkup population were treated as a normal control group. Transmission electron microscopy showing exosomes isolated from plasma samples of advanced metastasis group (AM-exo), early non metastasis group (ENM-exo), drug resistant group (DR-exo) and normal control group (NC-exo). **(B)** Western blot analysis of typical markers CD63 and HSP70 of exosomes isolated from four groups of plasma samples. **(C)** Flow cytometry analysis of exosomal tumor markers CK19, TAG72 and CA125 in exosomes isolated from four groups of plasma samples, CD63 used as exosomal marker control. **(D)** Exosomal tumor markers rapid detection by ELISA using exosomes suspension isolated from plasma samples of CRC patients. Presented data are representative of three independent experiments and are reported as means ± SD, $^{*}P < .05$.

exosomal CK19, TAG72 and CA125 were identified as markers for CRC. Exosomes derived CK19 was correlated with colorectal tissue. TAG72-rich exosomes indicated that CRC patients might be resistant to 5-FU. CA125-rich exosomes might be as metastatic CRC markers. These exciting discoveries have been continuously verified at the level of tumor interstitial fluid derived exosomes and patients' plasma derived exosomes. For these three markers, CK19 [18,30,31], TAG72 [32,33] and CA125 [34] are of guiding value for monitoring the occurrence and development of CRC.

In conclusion, this study showed for the first time that exosomal CK19, TAG72 and CA125 were used as special markers for CRC. This provided a potential prospect of cell exosomes as a marker for diagnosing disease. With the discovery of more exosomal tumor markers, humoral detection could play an important role in the diagnosis and monitoring of diseases.

Conflict of interest

The authors declare no conflict of interest.

Funding

This work was supported by grants from National Natural Science Foundation of China (81772277).

Acknowledgements

The authors thank the donors who provided their blood samples. The following is the supplementary data related to this article.

References

[1] X. Liu, X. Liu, Y. Wu, et al., MicroRNAs in biofluids are novel tools for bladder cancer screening, Oncotarget 8 (2017) 32370–32379.
[2] D.A. Raj, I. Fiume, G. Capasso, G. Pocsfalvi, A multiplex quantitative proteomics strategy for protein biomarker studies in urinary exosomes, Kidney Int. 81 (2012) 1263–1272.
[3] R.M. Hoffman, Clinical practice. Screening for prostate cancer, N. Engl. J. Med. 365 (2011) 2013–2019.
[4] M. Logozzi, D.F. Angelini, E. Iessi, et al., Increased PSA expression on prostate cancer exosomes in in vitro condition and in cancer patients, Cancer Lett. 403 (2017) 318–329.
[5] F. Properzi, M. Logozzi, S. Fais, Exosomes: the future of biomarkers in medicine, Biomark. Med 7 (2013) 769–778.
[6] E.G. Trams, C.J. Lauter, N. Salem, et al., Exfoliation of membrane ecto-enzymes in the form of micro-vesicles, Biochim. Biophys. Acta 645 (1981) 63–70.
[7] M. Yanez-Mo, P.R. Siljander, Z. Andreu, et al., Biological properties of extracellular vesicles and their physiological functions, J Extracell. Vesicl. 4 (2015) 27066.
[8] E. van der Pol, A.N. Boing, P. Harrison, et al., Classification, functions, and clinical relevance of extracellular vesicles, Pharmacol. Rev. 64 (2012) 676–705.
[9] T. Liu, X. Zhang, S.Y. Gao, et al., Exosomal long noncoding RNA CRNDE-h as a novel serum-based biomarker for diagnosis and prognosis of colorectal cancer, Oncotarget 7 (2016) 85551–85563.
[10] H.Y. Zhao, L.F. Yang, J. Baddour, et al., Tumor microenvironment derived exosomes pleiotropically modulate cancer cell metabolism, elife 5 (2016).
[11] F. Cappello, M. Logozzi, C. Campanella, et al., Exosome levels in human body fluids: a tumor marker by themselves? Eur. J. Pharm. Sci. 96 (2017) 93–98.
[12] M. Logozzi, A. De Milito, L. Lugini, et al., High levels of exosomes expressing CD63 and Caveolin-1 in plasma of melanoma patients, PLoS One 4 (2009).
[13] R. Xu, D.W. Greening, H.J. Zhu, et al., Extracellular vesicle isolation and characterization: toward clinical application, J. Clin. Invest. 126 (2016) 1152–1162.
[14] S. Kamerkar, V.S. LeBleu, H. Sugimoto, et al., Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer, Nature 546 (2017) 498–503.
[15] J.E. Celis, P. Gromov, T. Cabezon, et al., Proteomic characterization of the interstitial fluid perfusing the breast tumor microenvironment: a novel resource for biomarker and therapeutic target discovery, Mol. Cell. Proteomics 3 (2004) S27.
[16] J. Weitz, M. Koch, J. Debus, et al., Colorectal cancer, Lancet 365 (2005) 153–165.
[17] L.A. Torre, F. Bray, R.L. Siegel, et al., Global cancer statistics, 2012, CA Cancer J. Clin. 65 (2015) 87–108.
[18] R. Shimada, H. Iinuma, T. Akahane, et al., Prognostic significance of CTCs and CSCs of tumor drainage vein blood in Dukes' stage B and C colorectal cancer patients, Oncol. Rep. 27 (2012) 947–953.
[19] D.B. Longley, D.P. Harkin, P.G. Johnston, 5-fluorouracil: mechanisms of action and clinical strategies, Nat. Rev. Cancer 3 (2003) 330–338.
[20] A.H. Braun, W. Achterrath, H. Wilke, et al., New systemic frontline treatment for metastatic colorectal carcinoma, Cancer 100 (2004) 1558–1577.
[21] M. Colombo, G. Raposo, C. Thery, Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles, Annu. Rev. Cell Dev. Biol. 30 (2014) 255–289.
[22] J. Rak, Extracellular vesicles - biomarkers and effectors of the cellular interactome in cancer, Front. Pharmacol. 4 (2013) 21.
[23] A. Hoshino, B. Costa-Silva, T.L. Shen, et al., Tumour exosome integrins determine organotropic metastasis, Nature 527 (2015) 329–335.
[24] S. Zhang, Y. Zhang, J. Qu, et al., Exosomes promote cetuximab resistance via the PTEN/Akt pathway in colon cancer cells, Braz. J. Med. Biol. Res. 51 (2017) e6472.
[25] M. Bebawy, V. Combes, E. Lee, et al., Microparticles mediate transfer of P-glycoprotein to drug sensitive cancer cells, Leukemia 23 (2009) 1643–1649.
[26] R. Safaei, B.J. Larson, T.C. Cheng, et al., Abnormal lysosomal trafficking and enhanced exosomal export of cisplatin in drug-resistant human ovarian carcinoma cells, Mol. Cancer Ther. 4 (2005) 1595–1604.
[27] Y. Yuana, A. Sturk, R. Nieuwland, Extracellular vesicles in physiological and pathological conditions, Blood Rev. 27 (2013) 31–39.
[28] S. Fais, L. O'Driscoll, F.E. Borras, et al., Evidence-based clinical use of nanoscale extracellular vesicles in nanomedicine, ACS Nano 10 (2016) 3886–3899.
[29] C. Campanella, F. Rappa, C. Sciume, et al., Heat shock protein 60 levels in tissue and circulating exosomes in human large bowel cancer before and after ablative surgery, Cancer 121 (2015) 3230–3239.
[30] C. Alix-Panabieres, J.P. Vendrell, M. Slijper, et al., Full-length cytokeratin-19 is released by human tumor cells: a potential role in metastatic progression of breast cancer, Breast Cancer Res. 11 (2009) R39.
[31] M. Barone, D.F. Altomare, M.T. Rotelli, et al., Disseminated tumour cells in bone marrow in experimental colon cancer: metastatic or resident? Color. Dis. 15 (2013) 667–673.
[32] M. Swiderska, B. Choromanska, E. Dabrowska, et al., The diagnostics of colorectal cancer, Contemp. Oncol. (Pozn) 18 (2014) 1–6.
[33] S.P. Povoski, I.S. Hatzaras, C.M. Mojzisik, et al., Oncologic theranostics: recognition of this concept in antigen-directed cancer therapy for colorectal cancer with anti-TAG-72 monoclonal antibodies, Expert. Rev. Mol. Diagn. 11 (2011) 667–670.
[34] M.M. Streppel, A. Vincent, R. Mukherjee, et al., Mucin 16 (cancer antigen 125) expression in human tissues and cell lines and correlation with clinical outcome in adenocarcinomas of the pancreas, esophagus, stomach, and colon, Hum. Pathol. 43 (2012) 1755–1763.