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Reviewer A

In this review article, authors review various long RNAs that have been associated with extracellular vesicles in varios cancers and their utility. They include a description of EVs, their biogenesis, various kinds of RNAs identified in EVs followed by summary of EV-RNA based biomarkers for various cancers. In addtion they discuss the current challenges and future prospects. Following points should be addressed:

Comment 1: Line 46: Statistics from 2022 should be included.

Reply: Thanks for your advice. We have added Statistics from 2022 (see Page 3, line 47) .

Changes in the text: In 2022, approximately 4,820,000 and 1,918,030 new cancer cases, 3,210,000 and 609,360 deaths are estimated in China and the United States, respectively.

Comment 2: The section on biogenesis simply refers to various kinds of vesicles and does not include biogenesis. Please modify this section.

Reply: Thanks for your valuable suggestion. The biogenesis of different EVs were added in the section. (see Page 7, line 116) .

Changes in the text:

Although the classification of EVs is continuously changing, they are generally divided into three major groups, exosomes, microvesicles, and apoptotic bodies on the basis of their biogenesis and size. Exosomes, with a majority size of 30-100nm, originate from the inward budding of the endosomal membranes and the fusion of multivesicular bodies with the plasma membranes to secrete the membrane-encapsulated vesicles into the extracellular space. The Endosomal Sorting Complex Required for Transport (ESCRT) machinery is the most commonly described pathway for exosomal biogenesis. ESCRT-0 recognizes ubiquitinated proteins in the late endosomal membrane and initiates the process, ESCRT-I/II are then activated and drive the intraluminal membrane budding, and ESCRT-III is recruited via the programmed cell death 6-interacting protein and drives the shedding of vesicles. Finally, ESCRT system is disassembled by auxiliary proteins (specifically VPS4 ATPase). Microvesicles, ranging in size from 50nm to 1,000nm, their biogenesis is far less defined. In general, these vesicles are produced by the outward budding and fission of the plasma membranes, and then subsequently released into the extracellular environment. Unlike exosomes and microvesicles released by living cells, apoptotic bodies, with diameters of 0.5-2μm, are released from blebbing of the

plasma membrane of apoptotic cells or dying cells.

Comment 3: Full forms of gene symbols should be included.

Reply 3: Thanks for your advice. We have added the full names of genes throughout the manuscript.

Changes in the text:

Page 9, line 170	Y box binding protein 1 (YB-1) and NOP2/Sun RNA methyltransferase 2 (NSUN2)
Page 10, line 180	long intergenic non-protein coding RNA 858 (LINC00858)
Page 10, line 192	circular RNA homeodomain interacting protein kinase 3 (circHIPK3) and taurine up-regulated 1 (TUG1), urothelial cancer associated 1 (UCA1)
Page 12, line 235	brain expressed X-linked 2 (BEX2), AC104843.1, AL136981.2, keratin 19 (KRT19), nucleophosmin 1 pseudogene 25 (NPM1P25), cathepsin G (CTSG), carbonyl reductase 3 (CBR3), homeobox B7 (HOXB7), AL691447.3, RNA, 5S ribosomal pseudogene 141 (RNA5SP141)
Page 13, line 244	methylsterol monooxygenase 1 (MSMO1)
Page 13, line 252	ubiquitin-fold modifier conjugating enzyme 1 (UFC1), circular RNA ubiquitin specific peptidase 7 (circUSP7), NFKB inhibitor alpha (NFKBIA), NADH:ubiquinone oxidoreductase subunit B10 (NDUFB10), solute carrier family 7 member 7 (SLC7A7), actin related protein 2/3 complex subunit 5 (ARPC5), Septin 9 (SEPTIN9), high mobility group nucleosome binding domain 1 (HMGN1), H4 clustered histone 2 (H4C2)
Page 14, line 282	ribonuclease P RNA component H1 (RPPH1), carcinoembryonic antigen (CEA), carbohydrate antigen 199 and carbohydrate antigen 125
Page 15, line 304	metastasis associated lung adenocarcinoma transcript 1 (MALAT1), deleted in lymphocytic leukemia 2 (DLEU2), HOXA distal transcript antisense RNA (HOTTIP), and small nucleolar RNA host gene 1 (SNHG1), differentiation antagonizing non-protein coding RNA (DANCR)
Page 16, line 326	ETS transcription factor ERG (ERG), prostate cancer associated 3 (PCA3), and SAM pointed domain containing ETS transcription factor (SPDEF)
Page 17, line 358	fibrinogen alpha chain (FGA), KRT19, H2B clustered histone 12 (HIST1H2BK), inter-alpha-trypsin inhibitor heavy chain 2 (ITIH2), membrane associated ring-CH-type finger 2 (MARCH2), claudin 1 (CLDN1), mal, T cell differentiation protein 2 (MAL2) and TIMP metallopeptidase inhibitor 1 (TIMP1)

Table 2	ADAMTS9-AS1: ADAMTS9 antisense RNA 1, AUC: area under the curve, ARPC5: actin related protein 2/3 complex subunit 5, BCYRN1: brain cytoplasmic RNA 1, BEX2: brain expressed X-linked 2, CA9: carbonic anhydrase 9, CASP14: caspase 14, CBR3: carbonyl reductase 3, CCAT2: colon cancer associated transcript 2, CDC42: cell division cycle 42, circUSP7: circular RNA ubiquitin specific peptidase 7, CLDN1: claudin 1, CRC: Colorectal cancer, CTSG: cathepsin G, DANCR: differentiation antagonizing non-protein coding RNA, DLEU2: deleted in lymphocytic leukemia 2, ERG: ETS transcription factor ERG, ESCC: Esophageal squamous cell cancer, EV: extracellular vesicle, FGA: fibrinogen alpha chain, FRLnc1: Forkhead box protein M1 related long noncoding RNA, GAS5: growth arrest specific 5, GC: Gastric cancer, H4C2: H4 clustered histone 2, HCC: hepatocellular carcinoma, HIST1H2BK: H2B clustered histone 12, HMGN1: high mobility group nucleosome binding domain 1, HOTTIP: HOXA distal transcript antisense RNA, HOXB7: homeobox B7, HR: Hazard ratio, HULC: hepatocellular carcinoma up-regulated long non-coding RNA, IL32: interleukin 32, ITIH2: inter-alpha-trypsin inhibitor heavy chain 2, KLHDC7B: kelch domain containing 7B, KRT19: keratin 19, LINC00853: long intergenic non-protein coding RNA 853, LINC02418: long intergenic non-protein coding RNA 2418, lncRNA-GC1: gastric cancer associated long noncoding RNA1, SOX2OT: SOX2 overlapping transcript, MAL2: mal, T cell differentiation protein 2, MALAT1: metastasis associated lung adenocarcinoma transcript 1, MARCH2: membrane associated ring-CH-type finger 2, MAX: MYC associated factor X, MIR205HG: MIR205 host gene, MSMO1: methylsterol monooxygenase 1, NACT: Neoadjuvant chemotherapy, NCF2: neutrophil cytosolic factor 2, NDUFB10: NADH:ubiquinone oxidoreductase subunit B10, NFKBIA: NFKB inhibitor alpha, NPM1P25: nucleophosmin 1 pseudogene 25, NSCLC: Non small-cell lung cancer, NTA: Nanoparticle Tracking Analysis, PCA3: prostate cancer associated 3, PCAT6: prostate cancer associated transcript 6, PDAC: Pancreatic ductal adenocarcinoma, PDGFA: platelet derived growth factor subunit A, PRSS1: serine protease 1, PTENP1: phosphatase and tensin homolog pseudogene 1, RNA5SP141: RNA, 5S ribosomal pseudogene 141, RPPH1: ribonuclease P RNA component H1, SEPTIN9: septin 9, SLC7A7: solute carrier family 7 member 7, SLC9A3-AS1: SLC9A3 antisense RNA 1, SNHG1: small nucleolar RNA host gene 1, SRSF2: serine and arginine rich splicing factor 2, TEM: Transmission electron microscopy, TIMP1: TIMP metallopeptidase inhibitor 1, TMPRSS2: transmembrane serine protease 2, UFC1: ubiquitin-fold modifier conjugating enzyme 1

Comment 4: Line 355: infancy

Reply 4: Thanks for your advice. We have corrected it (see Page 19, line 393).

Changes in the text:

Although great progress has made, exLRs are still in infancy and rarely used in the clinic.

Reviewer B

The authors provide an informative overview of the potential of extracellular vesicle long RNAs (exLRs) for cancer diagnosis and prognosis. The authors have gathered relevant literature from PubMed to inform their review and discuss the current status and approaches of exLRs in early diagnosis and treatment response assessment for different types of cancer, such as breast cancer, lung cancer, hepatocellular carcinoma, prostate cancer, etc. They highlight the promise of exLRs as a tissue-specific and abundant liquid biopsy tool for cancer diagnosis and treatment monitoring.

Comment 1: The authors mentioned "one of the three major resources of liquid biopsy". It would be helpful if the authors could clarify what the three major resources of liquid biopsy are.

Reply 1: Thanks for your advice. We have added the information about the three major resources of liquid biopsy (see Page 4, line 62) .
Changes in the text: Circulating tumour cells, circulating tumour DNA and extracellular vesicles (EVs), are the three major resources of liquid biopsy.

Comment 2: The authors focused on long RNA; however, miRNA studies have also increased. It would be beneficial to provide a discussion on the significance of long RNAs over miRNA and why the authors focused on long RNAs in this review.

Reply 2: Thank you for your valuable advice. As you mentioned, the miRNAs studies increased. In fact, in the past decade, early investigations on EV-derived RNAs focused primarily on miRNAs. However, the clinical application of EV miRNAs is limited due to their low quantity and specificity. Vesiclepedia 2019 contained 2,431 unique miRNAs. And concerning the quantity of long RNAs, exoRBase database covered 19,643 mRNAs, 15,645 lncRNAs and 79,084 circRNAs.

From a liquid biopsy standpoint, the biomarkers used clinically are more typically long RNAs, especially the mRNA with known mutations and actionable alterations, for example, fusion transcripts, splice variants, represent alternative approaches that may not be detected using miRNAs. Thus, we focused on long RNAs in this review. (see Page 8, line 142) .
Changes in the text:
However, the clinical application of EV miRNAs is limited due to their low quantity (~2,500 EV miRNAs) and specificity. ...Nowadays, the exoRBase database has been updated to version 2.0 by gathering about 1,000 RNA-seq data of EVs from four types of human body fluids (blood, urine, CSF and bile), covering 19,643 mRNAs, 15,645 lncRNAs and 79,084 circRNAs.
Besides the abundant level of tumor specific biomarkers, long RNAs, can provide additional opportunities to study other processes that may indicate disease development/progression. For example, fusion transcripts (e.g., echinoderm microtubule-associated protein-like 4/anaplastic lymphoma kinase (EML4-ALK) fusion variant), splice variants (such as androgen receptor splice variant 7), represent alternative approaches that may not be detected using miRNAs, further promoting the research of exLRs.

Comment 3: The reference should be provided for the statement "Colorectal cancer is one of the most common malignancies worldwide and the second cause of cancer death worldwide."

Reply 3: Thanks for your advice. References for the statement of the colorectal cancer incidence and mortality were added (see Page 14, line 281).
Changes in the text:
Colorectal cancer is one of the most common malignancies worldwide and the second

cause of cancer death worldwide (1, 31).

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209-49.

31. Matsumura T, Sugimachi K, Iinuma H, et al. Exosomal microRNA in serum is a novel biomarker of recurrence in human colorectal cancer. Br J Cancer. 2015;113(2):275-81.

Comment 4: The authors should confirm whether the EV lncRNA in colorectal cancer section referred to is RPPH1 or RRPH1.

Reply 4: Thanks for your advice. We have modified the gene symbol to RPPH1, meanwhile, we checked other gene symbols throughout the manuscript (see Page 14, line 282) .

Changes in the text:

Liang et al indicated that EV lncRNA ribonuclease P RNA component H1 (RPPH1) was upregulated in CRC patients compared with healthy donors. Besides, EV RPPH1 showed a better performance than traditional tumor markers carcinoembryonic antigen (CEA), carbohydrate antigen 199 and carbohydrate antigen 125 in CRC diagnosis (AUC was 0.856, 0.790, 0.544 and 0.654, respectively), which suggested the potential of EV RPPH in CRC diagnosis.

Reviewer C

This review introduced the application of ExLR to cancer diagnosis and prognosis, presenting the issues and challenges that need to be addressed in EV research. Reviews of ExLR on oncology are relatively few. We are delighted to read such a good paper and thank the authors for their contribution. There are some minor suggestions as follows:

Comment 1: The manuscript fails to provide a persuasive rationale for publication this review in the introduction. The authors should have clarified what existing similar reviews have and have not summarized, before carrying out "In this review, we attempt to comprehensively review the literature on EVs, in particular, exLRs, from their characteristics to their potential applications in the diagnosis and prognosis of cancer."

Reply 1: Thank you for your suggestion. With the development of detection methods, large amounts of RNAs were detected in extracellular vesicles. The main reviews, however, revolves around the EV-derived miRNAs or non-coding RNAs (miRNAs, lncRNAs and circRNAs) and their contributions to diverses diseases.

Unlike other reviews, we focus the review on exLRs, including mRNAs, lncRNAs

and circRNAs, rather than EV-derived miRNAs or non-coding RNAs (miRNAs, lncRNAs and circRNAs). Besides, we focus their applications in oncology, rather than diverse diseases. In brief, in this review, we summarizes the biogenesis and characteristics of EV and exLRs, and highlights the current status and approaches of these biomarkers that have the potential to be used for early diagnosis and accurate prediction of therapeutic approaches. In addition, the prospects and challenges of applying exLRs to precision medicine are discussed. (See page 5, line 91).

Changes in the text:
With the growth of EV-related studies, novel diagnostic and prognostic biomarkers are emerging. Previous reviews have highlighted the characteristics and applications of EV-derived miRNAs or non-coding RNAs (including miRNAs, lncRNAs and circRNAs). In this review, we attempt to comprehensively review the literature on EVs, in particular, exLRs, from their characteristics to their potential applications in the diagnosis and prognosis of cancer. This review summarizes the biogenesis and characteristics of EV and exLRs, and highlights the current status and approaches of these biomarkers that have the potential to be used for early diagnosis and accurate prediction of therapeutic approaches. In addition, the prospects and challenges of applying exLRs to precision medicine are discussed.

Comment 2: The authors failed to explain why they choose the five categories of tumours to highlight in the "Clinical applications of exLR in cancer diagnosis and prognosis" section.

Reply 2:
Thank you for your comment. According to Global Cancer Statistics 2020, breast cancer is the most commonly diagnosed cancer, followed by lung, colorectal, prostate cancers. Lung cancer is the leading cause of cancer death, followed by colorectal , liver cancers. In addition, the application of exLR is relatively well established in prostate cancer and has been already in clinical use. Considering morbidity, mortality and clinical accessibility, we choose the five categories of tumours (breast cancer, lung cancer, colorectal cancer, liver cancer and prostate cancer) to highlight in the "Clinical applications of exLR in cancer diagnosis and prognosis" section. (See page 12, line 237).

Changes in the text:
Considering cancer morbidity, mortality and clinical accessibility, the applications of exLRs in breast cancer, lung cancer, colorectal cancer, liver cancer and prostate cancer are detailed below.

Comment 3: The key message is to some extent comprehensive. We recommend the authors give a brief summary in each subheading, especially including the overall

cutting-edge developments in the sub-areas and limitations of these findings.

Reply 3:
Thank you for your suggestions. Summaries were added in each subheading. (See page 13, line 257; page 14, line 290; page 15, line 310; page 16, line 330; page 18, line 360; page 19, line 381).

Changes in the text:
Breast cancer
In summary, the above evidences provide the proof of concept of exLRs as noninvasive diagnostic and prognostic markers for breast cancer. Despite great significance, more studies are needed to ensure the value of exLRs for future clinical use.

Lung cancer
Overall, the value of exLR detection contains cancer diagnosis and medication guidance. However, clinical significance remains to be explored in larger patient cohorts and diverse population.

Colorectal cancer
These suggested that exLRs have a higher diagnostic value of early-stage CRC than traditional tumor markers. However, further validation is needed to ensure the reproducibility of the results.

Hepatocellular carcinoma
To conclude, multi-marker exLR panels, in addition to single exLR marker candidates, could detect HCC patients in very stage and they could predict HCC recurrence. However, more research should be conducted to explore the potential application of exLRs in HCC diagnosis and treatment.

Prostate cancer
Altogether, these studies suggest that exLRs in blood or urine samples might be effective biomarkers to detect patients with prostate cancer, and urine-derived exLRs can be used for cancer discrimination from low-grade and benign disease population. However, the lack of standardized protocols for sample handling, which may affect reproducibility, is a limitation for clinical application.

Other tumors
In summary, exLRs are of diagnostic value for multiple cancers as they may provide additional information. However, there is still a long way to go for clinical use.

Comment 4: We kindly suggest that the authors change the order of the three separate subsections on EV-mRNA, EV-LncRNA, and EV-circRNA with the previous section - "Extracellular vesicle long RNA" - by first describing the biological features and functions and then referring to relevant studies in oncology, and need to adjust the content accordingly, which would further improve readability.

Reply 4:

Thank you for your suggestion. The arrangement may be easily confused, so we changed the style and added contents to improve readability.

The section on "Extracellular vesicle long RNA" introduces the history of EV RNAs and their respective basic concepts (contains only three separate subsections on EV-mRNA, EV-LncRNA, and EV-circRNA).

"Extracellular vesicle long RNA", “Characteristics of exLR”, “Clinical applications of exLRs in cancer diagnosis and prognosis” are three separate sections. (See page 9, line 172).

Changes in the text:

Extracellular vesicle long RNA

Thus in this review, we place our focus on the exLRs, including EV-mRNAs, lncRNAs and circRNAs.

EV-mRNA...

EV-lncRNA...

EV-circRNA...

Characteristics of exLR

Clinical applications of exLRs in cancer diagnosis and prognosis