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Exosomal circRNAs: new players in the field of cholangiocarcinoma

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Abstract

Cholangiocarcinoma (CCA) is a deadly cancer worldwide associated with limited therapeutic options. A recent study published in *Clinical Science* by Wang and colleagues [Clinical Science (2019) 133, 1935-1953] brought new perspectives to CCA management and therapy by focusing on circular RNAs (circRNAs). CircRNAs belong to an emerging class of functional non-coding RNAs (ncRNAs) regulating numerous biological processes. Notably, circRNAs have been associated with cancer onset and progression, although reports in CCA are very limited so far. In this work, the expression of circular RNA circ-0000284 (aka circHIPK3) was specifically elevated in CCA cell lines, human tumor tissues and plasma exosomes. Gain and loss of function approaches were performed to better understand the molecular mechanisms regulated by circ-0000284. Notably, the authors evaluated the role of circ-0000284 as a microRNA (miRNA) sponge. By prediction analysis and functional tests, a direct interaction was demonstrated with miR-637 that targets lymphocyte antigen-6 E (LY6E). Increased expression of circ-0000284 was associated with enhanced migration, invasion and proliferation of CCA cell lines. Interestingly, exosomal-mediated circ-0000284 was reported to exhibit pro-oncogenic effects on surrounding normal cells. Altogether, these

data not only highlight circRNAs as new players in CCA pathogenesis but also as promising molecules for innovative non-invasive biomarkers, as circRNAs are enriched and stable in exosomes. Further investigations on extracellular vesicles should provide the necessary tools to improve CCA diagnosis, and move towards targeted-therapies.

Keywords: Cholangiocarcinoma, circular RNA, exosomes, biomarkers

Comment on

Circ-0000284 arouses malignant phenotype of cholangiocarcinoma cells and regulates the biological functions of peripheral cells through cellular communication. [Clin Sci (Lond). 2019]

Introduction

Cholangiocarcinoma (CCA) refer to a heterogeneous group of aggressive tumors that arise within both intrahepatic and extrahepatic bile ducts. CCA represent the second most common cancer in the liver after hepatocellular carcinoma (HCC). CCA incidence has been steadily increasing during the last decades and the absence of characteristic symptoms restrains early diagnosis (1). In addition, resistance of CCA to conventional chemotherapies and radiotherapies approaches limits therapeutic options (2). Surgical approaches remain the best possibly curative alternative for a total recovery. However, even total resections with safety margins are associated with a high rate of recurrence and a 5-year survival ranging from 15% to 40% (3). Hence, fresh ideas aiming at identifying novel therapeutic strategies and biomarkers are urgently needed to improve the survival of patients with CCA.

circRNAs: an emerging class of functional ncRNAs

Recently, circular RNAs (circRNAs) emerged in the literature as a puzzling class of non-coding RNAs (ncRNAs). For a long time, circRNAs were viewed as splicing noise or byproducts of splicing errors. Eventually, they drew increasing interest after being found dysregulated in several types of cancer, including CCA (4). Subsequently, growing evidences indicated that circRNAs play a key role in cancer onset and progression, by regulating notably cell proliferation, migration or invasion (5). It is now recognized that circRNAs, unlike linear RNAs, are generated through back-splicing and form a covalently closed continuous loop without 3’ and 5’ extremities. This specific structure renders circRNAs more stable and resistant to exonuclease degradation. Hence, circRNAs could be of a valuable assistance for clinical diagnosis, possibly serving as potent predictive non-invasive biomarkers, notably in CCA (6). The origin, biogenesis, and functions of circRNAs remained unclear for many years. Nevertheless, points of consensus are starting to emerge in the literature. For instance, one of the latest studies in the field strengthened the established process governing circRNA biogenesis (7). Thus, it was confirmed that circRNAs are natural byproducts of spliceosome-mediated splicing. Notably, a model whereby intron definition, exon definition and back-

splicing require the same spliceosome machinery has been highlighted, suggesting that alternative back-splicing may compete with canonical splicing and thus regulate cell function at a post-transcriptional level. Interestingly, canonical and alternative back-splicing may be regulated by *cis* or *trans* factors (7). Accordingly, Conn et al. identified the TGFβ-induced RNA binding protein Quaking (QKI) as a regulator of circRNA biogenesis binding on introns flanking circRNA-forming exons (8).

Almost 30 years after the discovery of the first spliced circular products (9), active research in the field improved our understanding of circRNA function. Thus, circRNAs were mainly described as post-transcriptional regulators due to their capability to sponge microRNAs (miRNAs). However, additional functions have been attributed to circRNAs over the last years, including sequestration of proteins, competition with canonical mRNA splicing, and transcriptional regulation of their host gene (10). In addition, a new school of thought is reconsidering the non-coding nature of some circRNAs. This assertion is supported by recent evidences identifying translation initiation systems in circRNAs (11).

circRNAs: a wide-open field for research in CCA

To date, only few recent studies investigated the role of circRNAs in CCA pathogenesis. One of them focused on CDR1AS, a circRNA already largely described for its implication in cancer onset and progression. Thus, CDR1AS expression was significantly higher in CCA tissue samples and correlated with clinical features such as lymph node invasion, advanced TNM stage, and a poor overall survival (12). Another study reported the expression of circRNAs in intrahepatic cholangiocarcinoma (iCCA). Gene expression profiling using pangenomic microarrays identified TGFβ-induced long ncRNA TLINC (formerly known as CASC15*)* in iCCA cell lines. Subsequently, this work revealed the presence of circular isoforms of TLINC in iCCA tissue samples (13). Although the function of TLINC circRNA in CCA carcinogenesis was not explicitly explored, a putative role in cancer stem cell biology is supported by an elevated expression of TLINC circRNA in human embryonic stem cells. (14). A couple of studies performed by Xu et al. pinpointed two aberrantly expressed circRNAs in

both CCA cell lines and human tumors. The involvement of these circRNAs in CCA progression has been determined by both *in vitro* and *in vivo* experiments. Thus, circ-0001649 and circ-0005230 were respectively down- and up-regulated in CCA cell lines and human tumors and their role as putative miRNA sponge was evaluated. In both cases, these circRNAs were associated with CCA progression and deserve further investigations as therapeutic targets for CCA (4, 15). In fact, most of the studies reported in CCA so far highlighted circRNA deregulations and their clinical relevance rather than performing a deep and comprehensive functional analysis.

A comprehensive analysis of circ-0000284 (circHIPK3) in CCA

The recent study published in *Clinical Science* by Wang and colleagues identified for the first time in CCA, circ-0000284, an isoform of the well-known circHIPK3 circRNA involved in tumor progression (16). Prediction data reported 21 isoforms of circHIPK3 deriving from the same pre-mRNA (17). Although all these isoforms were extensively described in cancer, circ-0000284 is definitely the most investigated one (18-24). However, opposite actions for circ-0000284 were reported depending on the type of cancer (Table 1). Thus, circ-0000284 can exhibit either an oncogenic or a tumor suppressor role. As it stands, it is impossible to conclude firmly on a general function of circ-0000284 in cancer. However, it seems increasingly clear that circ-0000284 acts mainly as a miRNA sponge (Table 1). To this extent, there are strong evidences showing that circ-0000284 rather exhibits pro-oncogenic features. Further investigations are needed to determine whether specific circHIPK3 isoforms carry either oncogenic or tumor-suppressive features. As far as CCA is concerned, the recent work by Wang and colleagues demonstrated that circ-0000284 was able to sponge miR-637 and to promote CCA progression. Hence, the authors demonstrated that circ-0000284 acts as an oncogene by promoting CCA cell migration, invasion and proliferation. Further mechanistic experiments established the ability of circ-0000284 to sponge miR-637, leading to increased expression of lymphocyte antigen-6 E (LY6E) through a competitive binding to miR-637 (Figure 1). LY6 complex is a group of alloantigens that was first

discovered on lymphocytes. Interestingly, increased expression of LY6 family was reported in multiple types of cancer, and was subsequently associated with poor overall and disease free survival. Thus, high LY6E expression was significantly correlated with poor clinical outcome in colorectal cancer, glioma, ovarian cancer, gastric cancer, breast cancer and lung cancer. Altogether, these data suggest that LY6 family members play a crucial role in tumor progression and represent clinically relevant target (25). To date, these data are the first to support a correlation between a high LY6E expression and a pro-oncogenic action in CCA, and providing a noteworthy mechanistic explanation (16). However, LY6E is not the only target of miR-637 as reported in the literature (Table 2). Thus, miR-637 could affect CCA progression by targeting additional transcripts encoding proteins involved in cell proliferation and migration (e.g. CDK6, STAT3, AKT1) (Table 2). In addition, miR-637 could be itself regulated by non-coding endogenous competitors other than solely circHIPK3, as described in other diseases (26-34) (Table 2).

Exosomal circRNAs as non-invasive functional biomarkers in CCA

An important aspect of the study by Wang and colleagues relies on evaluating for the first time in CCA the influence of exosomal-transmited circRNAs on their immediate cellular microenvironment. Hence, autocrine and paracrine pro-tumorigenic actions mediated by exosomes-carried circRNAs (exo-circRNAs) were demonstrated (Figure 1). Although the elevated expression of exo-circ-0000284 has been measured in the host surrounding cells, no further information is provided as regard to the mechanisms that promotes cell migration and cell proliferation in these host cells. It was assumed that transformation of host cells was mediated by the same mechanism than the one described in tumor cells where exo-circ-0000284 was originating from. Thus, a mechanism involving miR-637 sponging needs to be more clearly defined. In addition, the possibility that exo-circ-0000284 involves different miRNA regulatory networks in host cells should not be excluded. Interestingly, an increased expression of circ-0000284 was also shown in plasma-derived exosomes from patients with CCA. To our knowledge, this is the first report of circulating exo-circRNAs in CCA. Thus,

these findings provided a promising alternative for existing CCA biomarkers. Similarly, a recent paper highlighted the clinical relevance of protein biomarkers conveyed in body fluids via extracellular vesicles (EVs) in CCA (35). Thus, CCA-derived EVs from patient serum might serve as cargo for oncogenic proteins (35). Similar to circRNAs, EV-derived biomarkers are about to progressively challenge the poor established biomarkers in CCA. For instance, serum level of carbohydrate antigen 19-9 (CA19-9) or carcinoembryonic antigen (CEA) are two possible biomarkers to suspect CCA diagnosis. However, several studies reported the poor specificity and sensitivity of these biomarkers, highlighting the need for more efficient substitutes for CCA diagnosis and prognosis (36-38). For that purpose, it would be of great interest to identify clinically relevant circRNA signatures in CCA to improve patient outcome, as circRNAs are easily detectable and stable in body fluids.

Conclusion

In conclusion, recent data provide evidence of circRNAs potential as robust biomarkers with diagnostic and prognostic values in CCA. Remarkably, Wang and colleagues identified, for the first time in CCA, an exosome-mediated circRNA that was able to display autocrine and paracrine actions promoting CCA progression. Interestingly, this circRNA was also found in plasma-derived-exosomes, opening new perspectives for the use of exo-circRNAs as non-invasive biomarkers. Further investigations should provide the necessary tools to improve CCA diagnosis, and move towards targeted-therapies.

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Conflicts of interest

None.

Abbreviations: CCA, cholangiocarcinoma; circRNAs, circular RNAs; EVs, extracellular vesicles; exo-circRNAs, exosomes circRNAs; HCC, hepatocellular carcinoma; LY6E, lymphocyte antigen-6 E; miRNAs, microRNAs; ncRNAs, non-coding RNAs.

Figure legend:

Figure 1: Circ-0000284 promotes tumor progression in CCA cells acting as competitive endogenous RNA by sponging miR-637. Circ-0000284 holds sway over surrounding cells biological functions through exosomal communication. Exosomes released in blood represent a potential biomarker for CCA.

(modified from Servier Medical images: https://smart.servier.com).

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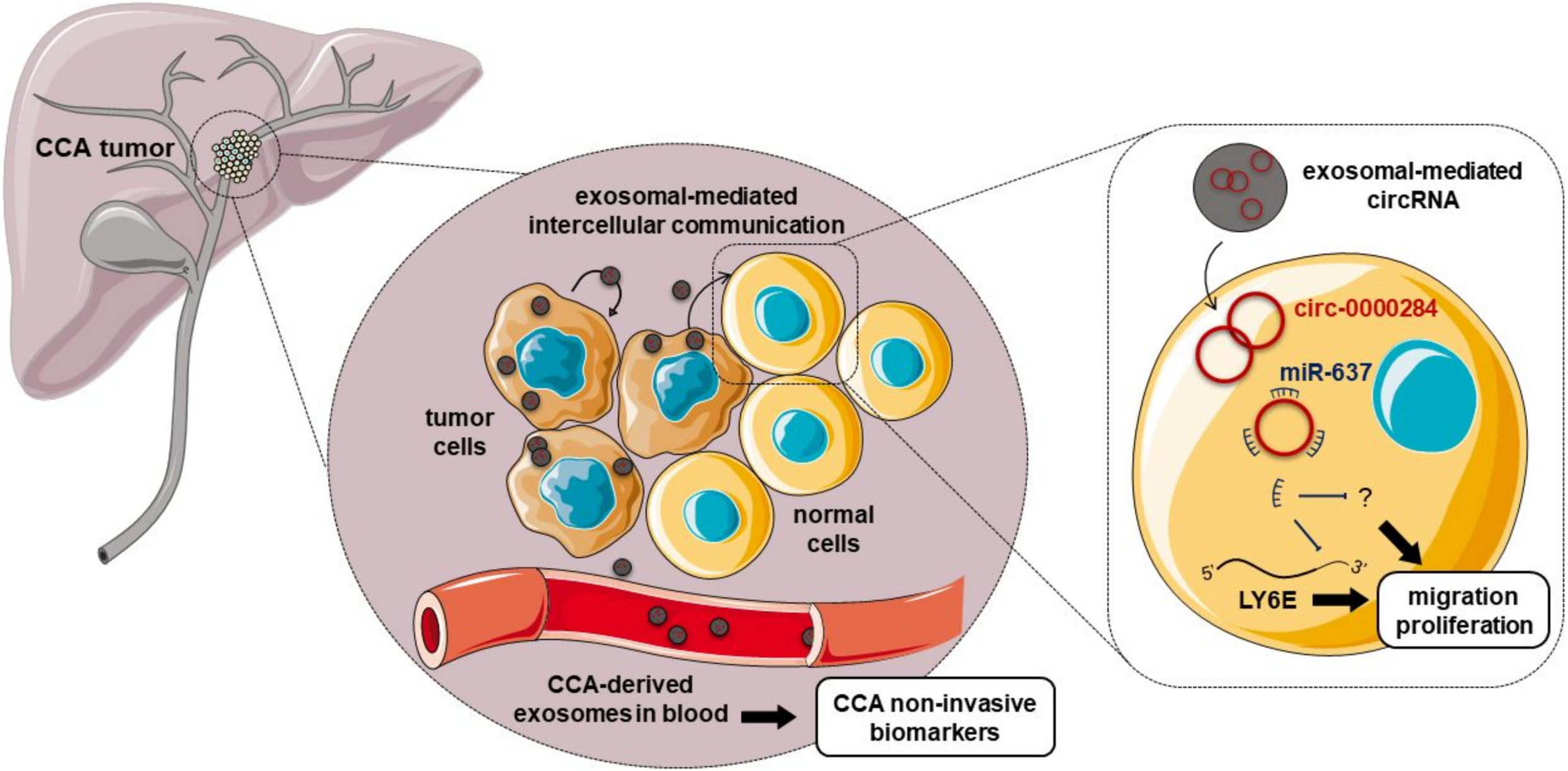

CCA tumor
exosomal-mediated
intercellular communication
tumor
cells
normal
cells
CCA-derived
exosomes in blood
CCA non-invasive
biomarkers
exosomal-mediated
circRNA
circ-0000284
miR-637
?
5'
3'
LY6E
migration
proliferation

Table 1: Examples of cancer-specific role and regulatory functions of circ-000284

Cancer type	Function	Oncogene or Tumor suppressor	Reference
Bladder cancer	miRNA sponge (miR-558)	Tumor supressor	[23]
Colorectal cancer	miRNA sponge (miR-7)	Oncogene	[24]
Hepatocellular carcinoma	miRNA sponge (miR-124)	Oncogene	[20]
Glioma	miRNA sponge (miR-654)	Oncogene	[21]
Nasopharyngal carcinoma	miRNA sponge (miR-4288)	Oncogene	[22]
Cholangiocarcinoma	miRNA sponge (miR-637)	Oncogene	[16]

Table 2: Examples of disease-specific targets and regulatory functions of miR-637.

miR-637 target	Endogenous miR-637 competitor	Disease	Reference
CDK6	LINC00473	Glioma	[26]
KLK4	circCEACAM5	Ovarian cancer	[27]
RING1	C5orf66-AS1 lncRNA	Cervical cancer	[28]
STAT3	circHIPK3	Colorectal cancer	[29]
NUPR1	FAL1 lncRNA		[30]
	LINC01234	Oral squamous cell carcinoma	[31]
APELIN	circNOTCH1	Gastric cancer	[32]
MMP19	circERBB2		[33]
AKT1	FAL1 lncRNA	Hirschsprung's disease	[34]