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# Are the Functions of Milk Exosomes Restricted to Their Protein Cargoes?

Elrashdy M. Redwan
*King Abdulaziz University*

Vladimir N. Uversky
*University of South Florida*, vuversky@usf.edu

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Numerous studies have analyzed the benefits of camel milk health over human and bovine milk [1–3]. These health benefits range from broad antimicrobial and antiviral potentials to anti-cancer activity and capability to act as anti-diabetics. The milk ingredients behind these benefits are variable and can be changed depending on the lactation season. To explore which components are responsible for these benefits, reports are often focused on the analysis of various biological macromolecules, but most studies are concentrated on the milk proteome analysis, whereas many other important biomolecules that can be found in milk, such as RNA, polysaccharides, and/or lipids, are mostly ignored in such proteome-centric studies. Noticeable variations are also observed between different studies, with outputs showing strong dependence on the evaluation methods used. Furthermore, recent studies become increasingly focused on the analysis of extracellular vesicles (EVs), such as milk fat globules (MFGs) and/or exosomes, rather than at the entire set of milk constituents. A recently published article by El-kattawy et al. [4] represents an example of such a protein-centric study, where the authors ascribed the *in vitro* anti-cancer effects of the exosomes extracted from camel milk to the lactoferrin and κ-casein proteins.

Milk exosomes contain many proteins with single or multiple functions, lipids, mRNAs, and numerous microRNAs (miRNAs, which is a large family of ~22 nucleotide (nt) non-coding small RNAs derived from the ~ 70 nt long stem-loop precursors (pre-miRNAs)) [5], as well as long non-coding RNAs (lncRNAs), circular RNAs (circRNAs) [6], and oligosaccharides and polysaccharides [7]. For example, in one study it was reported that human breast milk exosomes contain 602 unique miRNAs [8]. The authors emphasized that these exosomal miRNAs were resistant to various harsh conditions [8]. Since among these unique miRNAs found in exosomes 59 originated from the well-characterized immune-related pre-miRNAs, it was hypothesized that being transferred from the mother's milk to the infant, these exosomal miRNAs may be related to the development of the infant immune system [8]. In addition, exosomal long lncRNAs and circRNAs, which are abundantly present in milk exosomes (*e.g.*, in porcine milk exosomes, 2466 novel lncRNAs, 809 annotated lncRNAs, and 61 circRNAs were recently identified [6]), can be absorbed in the mammalian intestinal tract and become involved in regulation of gene expression [6]. Furthermore, the authors pointed out that there is an important interplay between the exosomal lncRNA/circRNA and miRNA [6]. As far as milk oligosaccharides are concerned, a recent study indicated that milk exosomes encapsulating human milk oligosaccharides (HMOs) were uptaken by macrophages responsible for the intestinal immunity establishment and modification of the newborn innate immunity [7].

Furthermore, milk exosomes are also characterized by a very interesting lipid content, in addition to their phospholipids bilayers. Importantly, many milk fat components that can be found within milk EVs (such as butyric acid, conjugated linoleic acid, phospholipids, and sphingolipids from MFGs) are known as potent anticarcinogenic agents [9].

Besides lactoferrin, κ-casein, camel milk contains α–lactalbumin and lactadherin. Lactadherin (also known as milk fat globule-epidermal growth factor-8, MFG-E8) is a 53-kDa glycoprotein, which is present in exosome-like vesicles (100–200 nm) secreted by mammary epithelial cells. Its expression in the mammary gland is considered crucial for the secretion of the milk lipids, especially in the form of small lipid globules, MFGs, which are typically below 1 μm in diameter, and are released as exosomes in an exocytic manner or as microvesicles originating from the membrane shedding [10–13]. Lactadherin/MFG-E8 exerts many functions, which include an important scavenging role against cancer [13]. α–Lactalbumin, a small globular $Ca^{+2}$ binding protein, has a decisive role in the final step of the lactose biosynthesis within the Golgi membranous compartment, where it can be incorporated into the exosomes, which are small unilamellar vesicles (SUV) of dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC). Furthermore, the *in vitro* analysis showed that this incorporation can also be expanded to other types of phospholipids.

The conformations of the membrane-bound form of α–lactalbumin range from native-like to molten globule-like states [14]. Although the incorporation of both α–lactalbumin and oleic acid into the camel milk EVs including exosomes is elusive, the prominent anticancer effects of α–lactalbumin-oleic acid complexes are studied very well, especially for the α–lactalbumins of human, bovine, and camel origin. In fact, when α–lactalbumin is naturally or artificially formulated with oleic acid, it yields human/bovine/camel α-lactalbumin made lethal to tumor cells (HAMLET, BAMLET, and CAMLET, respectively) preparations, which exert these anticancer activities [15–17]. The first signs of this discovery came from the observation of the selective effects of whole milk against

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certain types of carcinoma cell lines [18]. The active component was isolated from the casein fraction of milk and was identified as a multimeric α–lactalbumin (MAL) complexed with oleic acid [18]. Subsequent studies revealed that the actual tumoricidal activity is defined by the oleic acid [19], and many different proteins can be converted to liprotides, cytotoxic protein-oleic acid complexes, where lipids are bound to partially denatured proteins [20–22].

For almost 90 years it has been known that camel milk contains large amounts of oleic acid, which accounts for 38.9% (weight percentage) of overall fatty acid composition [23]. These levels markedly exceed the oleic acid contents of human or bovine milk (~ 23.8%) [24]. Furthermore, noticeable difficulties associated with the extraction of camel milk fat by traditional methods were attributed to the tendency of fatty acids to be bound to some camel milk proteins [25]. All these observations raised several important questions: can these high natural levels of oleic acid found in camel milk be responsible for its anticancer activities *in vitro* and/or *in vivo*? Can at least some of this highly abundant monounsaturated fatty acid be incorporated into the EVs or exosomes during their secretion? In fact, it is likely that the oleic acid bound to α–lactalbumin, lactadherin, lactoferrin, or other milk proteins found on or within the EVs and/or exosomes might have pivotal roles in the functions of these vesicles.

Therefore, it is likely that the functions of milk EVs and/or exosomes are not limited to their proteins, and other exosome ingredients (*e.g.*, RNA, polysaccharides, and lipids including oleic acid) are also important.

## Conflict of Interest

The authors declare that there are no conflicts of interest.

## Data availability

No data was used for the research described in the article.

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Elrashdy M. Redwan[a], Vladimir N. Uversky[b,*]
[a] *Biological Science Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia*
[b] *Department of Molecular Medicine, University of South Florida, Tampa, FL, United States*

[*] Corresponding author.
*E-mail addresses:* lradwan@kau.edu.sa (E.M. Redwan), vuversky@usf.edu (V.N. Uversky).