ORIGINALIA

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EXOSOMES, THE SHUTTLES OF INTERCELLULAR COMMUNICATION

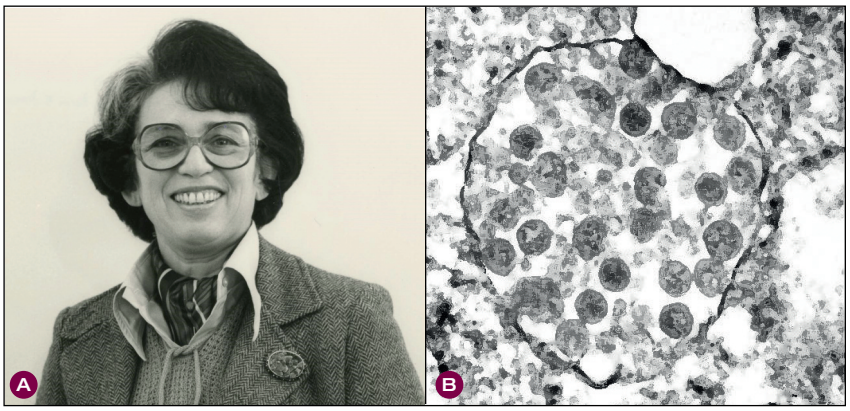
– THEIR EMERGING ROLE AND DIAGNOSTIC AND THERAPEUTIC POSSIBILITIES

When a wise man points at the moon,
the fool looks at the finger.

THE SHEEP, THE RAT AND "ALICE IN BLUNDERLAND"

In her historical review article published in Cell, May-June 2005 (Johnstone, 2005), a few years before her death and about 20 years after her discovery of **exosomes** and their expulsion from the plasma membrane of mature sheep reticulocytes (Pan & Johnstone, 1983), **Rose Johnstone**, born Mamelak, of Polish origin (Lodz-Poland, 1928 – Montréal-Canada, 2009), ironically defines the entire research journey that led to her crucial discovery as Alice in a "Land of Errors" *(Alice in Blunderland, an Iridescent Dream)*, referring, in the first part of the title of a paragraph, to the title of the famous short story by John Kendrick Bangs (1907), a parody of the better known "Alice's Adventures in Wonderland" by Lewis Carroll (1865).

– The word *"blunder"* means error, misunderstanding, misstep, mistake, to underline the many difficulties and unforeseen events she encountered while carrying out of her experiments and those of her young Chinese collaborator **BinTao Pan** in the Department of Biochemistry of the Faculty of



A – Rose Mary Johnstone pictured at the time of her discovery of exosomes in 1983 (McGill University Historical Archives, Montreal – Canada).

B – Multivesicular body (MVB) containing approximately thirty intraluminal vesicles (ILVs).
The membrane of the MVB fuses with the plasma membrane of the mother cell, thus releasing ILVs.
– These, having reached the extracellular space, are termed exosomes (EXO).

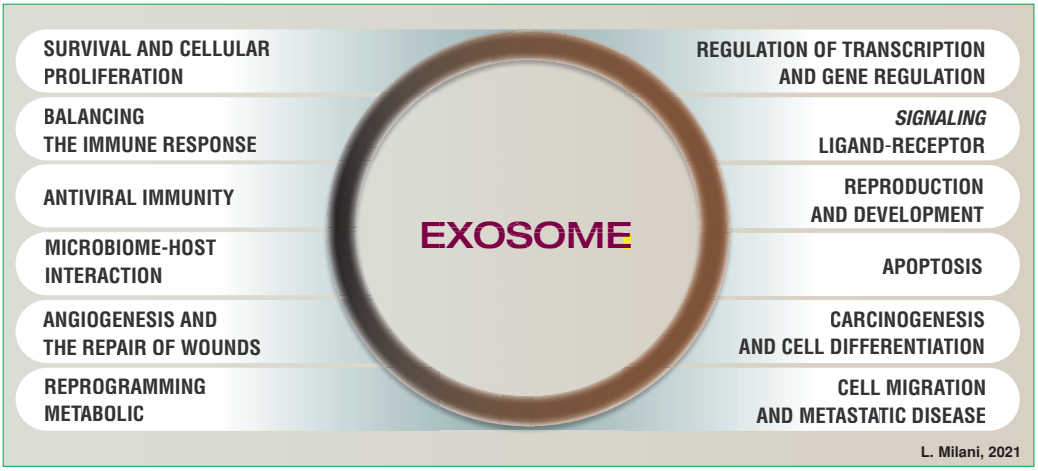


FIG. 1

Main physiological and pathological activities performed by exosomes.

Medicine at McGill University in Montreal Canada, and to highlight the fact that, at the time of the discovery and for many years to follow, exosomes were considered nothing more than "waste", "refuse", cellular "garbage" to be eliminated from the cytoplasm. Surely an unusual, original title for a paragraph, full of the many inevitable unknowns and errors involved in lengthy research and a key biological discovery under the electron microscope (SEM).

– Less than one week [at the very same time (Harding *et* Al., 2013)] after Pan & Johnstone's publication, the *Journal of Cell Biology* published a paper by **Harding**, **Heuser** & **Stahl** (1983) on their research at the University of Washington in St. Louis USA, in which some tiny vesicles ejected (*jettisoned* in the original English = thrown out) into the extracellular space by the mature reticulocytes of rats are photographed under a SEM.

• The term *"exosome"* is mentioned in neither Pan & Johnstone's article nor that of Harding *et* Al.; it was coined 4 years later by Professor Johnstone (Johnstone *et* Al., 1987), although the same name had been used 6 years earlier in reference, however, to some isolated cell membrane fragments in body fluids (Trams *et* Al., 1981) which, strictly speaking, had nothing to do with exosomes.

It should be noted that the Johnstone and Stahl research groups were independent, and that both were conducting experiments on the formation and internalisation of transferrin receptors in mature reticulocytes.

The discovery of the production of tiny nanoparticles (thereafter termed exosomes) expelled into the extracellular space by reticulocytes (young erythrocytes) is to be considered completely unexpected and purely fortuitous.

– Although the paternity (or rather: maternity) of the discovery of **exosomes (EXO)** is still debated today (Harding *et* Al., 2013), it is generally attributed to Johnstone, for two reasons; her publication pre-dates that of Harding et Al., even if only by a few days, and the term "exosome" was revived by the brilliant Canadian researcher in reference to what she had discovered 4 years earlier.

• Rose M. Johnstone was the first and only woman to hold the Chair of Biochemistry in the Faculty of Medicine at the McGill University of Montreal-Canada.

She is also remembered as a highly active promoter of women's rights in the academic and scientific world, a commitment she maintained with continuity and determination even after retirement, until her death at the age of 81 years.

– As mentioned above, EXOs were initially considered as by-products of cellular metabolism and their role went unrecognised: that they had a significant impact even on the cells close to the mother cell.

In fact, from 1983 (the year of the discovery) to 2000, the number of publications on EXOs was extremely small; only in the early years of the new millennium, about 20 years **after** their discovery, did researchers focus their attention on EXOs and interest literally exploded.

• There are now approximately 8500 publications, 2 dedicated scientific societies (American Society for Exosomes and Microvesicles and International Society for Extracellular Vesicles), a specialised journal [Journal of Extracellular Vesicles (impact factor 2019: 14.976)], 2 databases (Exocarta and Vesiclepedia), and one research portal (exRNA).

EXOSOMES – THE MYSTERIOUS MESSENGERS

EXOs are tiny microvesicles (nanoparticles) that tend to be rounded or oval shaped, with a **diameter** of **50-100 nm*** generated by the membrane of the intracellular multivesicular

* **1 nanometre (nm)** = 1 x 10^{-9} m = 1 billionth of a metre = 10 ångström.
In the past the nanometer was called a millimicron (1/1000 micron).
On average, an exosome is 1000 times smaller than the cell that generates it.
– One million exosomes could fit onto the tip of a pencil.

bodies (see page 33, FIG. B and FIG. 3) through the process of expulsion into the extracellular space, genuine shuttles carrying a load of proteins (Simpson *et* Al., 2009), lipids (Vidal *et* Al., 1989), and nucleic acids (Valadi *et* Al., 2007; Waldenstrom *et* Al., 2012) able to "deliver" their contents to the recipient cells they encounter, and reprogramme them.

EXOs are, and represent, a new method of **intercellular communication** which plays a key role in many cellular processes, for example the immune response, the first to be identified (Raposo *et* Al.,1996; Greening *et* Al., 2015), signal transduction (Gangoda *et* Al., 2015), antigen presentation (Mittelbrunn *et* Al., 2011) and many others (Fernandes *et* Al., 2020) (FIG. 1).

– EXOs can be released by virtually **all** eukaryotic cells (Ruivo *et* Al., 2017), cells with a defined nucleus, isolated from the remaining the cytoplasm by a membrane.

Recently, EXOs have also been photographed after their expulsion from prokaryotic cells (Kalluri & LeBleu, 2020).

• EXOs are generated by all **animal** and **plant cells**; by varying their load according to the type of cell from which they are derived, EXOs can provide – among other things – valuable prognostic information in a very wide range of diseases, such as: **1)** chronic inflammation (Lesser *et* Al., 2016; Hessvik & Llorente, 2018); **2)** diseases of the lipid metabolism (Record *et* Al., 2014); **3)** cardiovascular diseases (Kishore *et* Al., 2016); **4)** liver diseases (Sung *et* Al., 2018); **5)** kidney diseases (Gonzalez-Calero *et* Al., 2014); **6)** neurodegenerative diseases (Howitt & Hill, 2016); **7)** cancer (Salem *et* Al., 2016), and others.

EXOs have been isolated from various human body "fluids", for example blood (Caby *et* Al., 2005), urine (Pisitkun *et* Al., 2004), saliva (Ogawa *et* Al., 2011), sweat (Wu *et* Al., 2018), sperm (Ronquist & Brody, 1985; Park *et* Al., 2011; Aalberts *et* Al., 2012), milk (Admyre *et* Al., 2007), amniotic fluid (Asea *et* Al., 2008), cerebrospinal fluid (Vella *et* Al., 2008), ascitic fluid (Andre *et* Al., 2002), and bile (Masyuk *et* Al., 2010).

– These EXOs, with specific genomic, protein and lipid profiles, reflect the cellular origin as "fingerprints" or "signatures" of the cells that generated them and, as a result, have great potential use as biomarkers of many diseases (see below).

Although biological fluids are relatively simple to obtain ("liquid" biopsies) and rich in EXOs, these have not yet been used as biomarkers in current clinical practice (Zhang *et* Al., 2019).

It is only a matter of a few years, since dedicated studies are developing exponentially all over the world. Having one or more biomarkers of a disease before it becomes clinically evident means directing its course and prognosis.

EXOs derived from neoplastic cells contain RNA that can be incorporated into healthy cells, thus promoting the spread of the primary tumour (Skog *et* Al., 2008).

Although most research has been carried out on cells of mammalian species, some have shown that strains of *Leishmania* and *Mycobacterium* are also able to generate EXOs and thus regulate host defence (Silvermann *et* Al., 2010).

– In a similar way, this "pathway" enables the dissemination of molecules used by pathogens in an attempt to subvert the host's immune response and enable infections/infestations to persist or make them difficult to resolve.

In humans, as in parasites, the proteins contained in the exosomal cargo and transported some distance away remain intact and maintain their catalytic activity like the native

	EXOSOMES	MICROVESICOLES	ECTOSOMES	MEMBRANE PARTICLES	EXOSOME-LIKE VESICLES	APOPTOTIC VESICLES
ORIGIN	ENDOSOMES	PLASMA MEMBRANE	PLASMA MEMBRANE	PLASMA MEMBRANE	INTERNAL COMPARTMENTS	ND
DIMENSIONS	**50-100 nm**	**100-1000 nm**	**50-200 nm**	**50-80 nm**	**20-50 nm**	**50-500 nm**
SEDIMENTATION	100,000 g	10,000 g	160,000-200,000 g	100,000-200,000 g	175,000 g	1,200 g, 10,000 g or 16,000 g
LIPIDIC COMPOSITION (partial)	CHOLESTEROL SPHINGOMYELIN CERAMIDES EXPOSED PHOSPHATIDYLSERINE	EXPOSED PHOSPHATIDYLSERINE	CHOLESTEROL DIACYL-GLYCEROL EXPOSED PHOSPHATIDYLSERINE	ND	DO NOT CONTAIN LIPIDS	ND
MAIN PROTEIN MARKERS	TETRASPANINS (CD63, CD9) ALIX TSG 101	INTEGRINS, SELECTINS	PROTEOLYTIC ENZYMES NO CD63	NO CD63	TNF RECEPTOR (TNF R1)	HISTONES

TAB. 1

Physical and chemical characteristics of different types of vesicles discovered in the extracellular *milieu*.
– Apoptotic vesicles are only found in the extracellular *milieu* following apoptosis of a cell; ND = not defined.

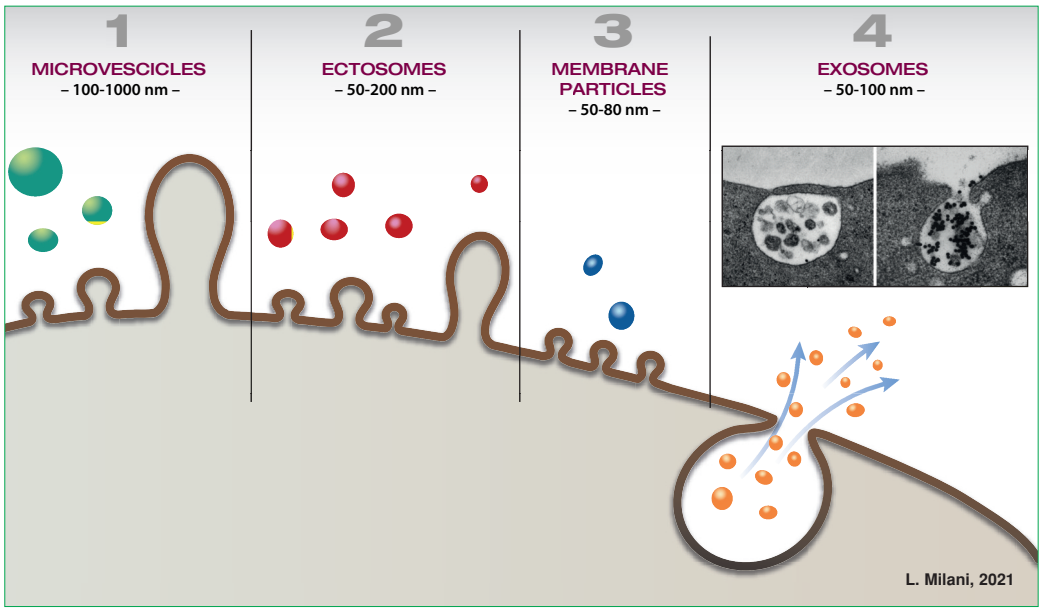
– Adapted from Javalet, 2016, adapted from Théry, 2009 (see References).

FIG. 2

Microvesicles (1), ectosomes (2), and membrane particles (3) result from a budding (exvagination) of the cell membrane and mainly contain the cytosol of the cell that generated them.

Exosomes (4) are – instead – released directly into the extracellular space thanks to an invagination of the cell membrane, passing – as a result – through a gap in the cell membrane which, subsequently, closes again.

– Each exosome, like 1, 2, and 3, has a double membrane.



ones (they are the same!), modifying the physiology and fate of the recipient cells (Colombo *et* Al., 2013; Kalra *et* Al., 2016).

• This intercellular communication physiologically enables the harmonious coordination of an organism's cellular functions as a whole, and thus promotes broader functional homeostasis as per Cannon (Cannon, 1929) and the allostatic state as per Sterling & Eyer (Sterling & Eyer, 1988).

BIOGENESIS OF EXOSOMES

To clarify the terminology and correct a few mistranslations or dubious interpretations, it is important to specify that cells secrete different types of extracellular vesicles: the first 3 are formed from a **budding** of the plasma membrane (**microvesicles, ectosomes,** and **membrane particles**) (TAB. 1; FIG. 2).

• On the other hand, EXOs have an **intracellular origin** and are released into the extracellular space by fusion of a mature multivesicular body with the cell membrane which, after expelling the EXOs, closes.

It never requires a continuous solution between the cytoplasm and extracellular "fluid".

There are also some recent descriptions of so-called EXO-like structures (see TAB. 1) of intracellular origin, whose function has still not been clearly defined (Fernades *et* Al., 2020; Lu & Huang, 2020).

– Briefly and chronologically, the formation of EXOs basically takes place in **4 stages** (FIG. 3):

• **Stage 1**: formation per **internal budding** of the cellular membrane of a small endocytic vesicle.

• **Stage 2**: enlargement of the endocytic vesicle, forming an **endosome** that generates a few small intraluminal vesicles **(ILV)** inside it.

• **Stage 3**: formation of a multivesicular body **(MVB)**.

MVBs are therefore formed during the maturation of the endosome (see Stage 2), a process through which the ILVs (see Stage 2) are generated by invagination of the bordering membrane. MVBs had already been observed precisely 20 years before the discovery of EXOs (Fuchs, 1963; Policard *et* Al.,1963) but, again, no biological significance had been attached to them.

– At this point, the MBVs can meet 2 fates: either they **3a)** merge with a lysosome, a cell organelle containing lytic enzymes that can break down organic components (digestive system of the cell) or they **3b)** migrate towards the cell membrane. The proteins and/or lipoprotein complexes necessary to the transport of MVBs inside the cell have recently been identified (Hurley & Hanson, 2010; Hanson *et* Al., 2012; Henne *et* Al., 2012).

• **Stage 4**: fusion of the MVBs with the cell membrane and release of ILVs into the extracellular space; from this point on, the ILVs are defined as EXOs.

From Endosome to EXOs, progressive invaginations of the plasma membrane, forming more and more, and smaller and smaller elements, are performed by the so-called ESCRT (Endosomial Sorting Complex Requiring for Transport) machinery, which is made up of 4 multiprotein complexes (0-I-II- III) (FIG. 4).

Intracellular Calcium is the true regulator of the fusion of MVB to the cell membrane, leading to the expulsion of EXOs (Savina *et* Al., 2003; 2005). This role is aided by the ALIX protein (Baietti *et* Al., 2012), whose three-dimensional structure has been determined (Fisher *et* Al., 2007; Larios *et* Al., 2020).

– This dynamic model, whose main points were formulated and published by Harding, Heuser & Stahl in 1983, has withstood the test of time, as claimed by one of the 3 authors, Philip Stahl "a distinguished plenary speaker" who had coordinated the experiment at the time, in a 2014 Congress organised by ISEV (International Society for Extracellular Vesicles) in Rotterdam The Netherlands and, more recently, confirmed in one of his publications (Stahl & Raposo, 2019).

EXOSOMES – THE *CARGO*

At each stage, from endocytic vesicle to EXO, selected quantities of various types of RNA, proteins and lipids are loaded into it.
Approximately 4400 proteins, 200 lipids, 1700 types of messenger RNA **(mRNA)** and 800 microRNA **(miRNA)** have so far been identified in the various EXOs examined (Mathivanan *et* Al., 2012; Kim *et* Al., 2013).

– Of particular interest is the role played by RNAs, powerful gene regulators. Indeed, RNA contained in EXOs **can modify the gene expression of the recipient cells** (Valadi *et* Al., 2007; Nolte-'t Hoen *et* Al., 2012).
The various types of RNA are not coding inside EXOs, but they certainly interfere with the RNA of the recipient cells.
This is how tumours metastasise remotely (Minciacchi *et* Al., 2015). This is how EXOs produced by the healthy cells of a specific organ regenerate diseased cells of that organ in a patient **(exosomal regenerative therapy)**.

EXOs basically contain 5 types of miRNA:
1) MicroRNAs (miRNA) - which consists of 20-22 nucleotides. These miRNAs have been proposed as non-invasive biomarkers, for example of cholangiocarcinoma (Puik *et* Al., 2017), small cell lung cancer (Hu *et* Al., 2020) and non-small cell lung cancer (Grimolizzi *et* Al., 2017).
miRNAs represent 76% of all map readings; they are transferred to the recipient cells, resulting in a transient or persistent phenotypic modification (Mittelbrunn *et* Al., 2011), and genotypic modification through RNA-dependent DNA polymerase = reverse transcriptase = retrotranscriptase.

2) lncRNA - consisting of 200 nucleotides involved in the regulation of cell differentiation, the cell cycle and epigenetics (Hu *et* Al., 2015), angiogenesis, metastasis, and neoplastic growth (Hewson & Morris, 2016).

3) CirRNA (circular RNA), a biomarker with diagnostic, prognostic, predictive properties.

4) Messenger RNA (mRNA).

5) other types of RNA (long non-coding RNA; piwi interacting RNA, transfer RNA, small nuclear RNA, small nucleolar RNA).

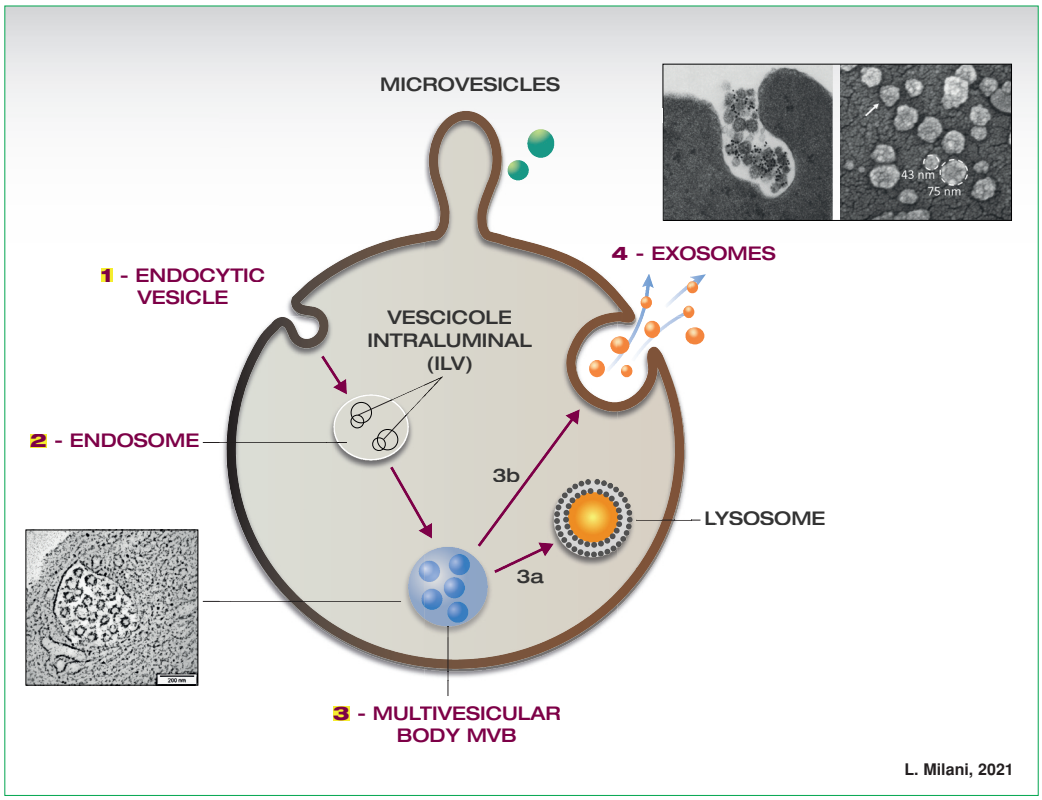


FIG. 3

Generation of exosomes and microvesicles.

– Multivesicular bodies (MVB) (3) are formed during the maturation of endosomes (2), a process through which the intraluminal vesicles (ILV) are generated by invagination of the bordering membrane of the endosome.
MVBs can fuse with lysosomes (3a) in order to break down their contents, or (3b) fuse with the cell membrane, releasing ILVs which, once they have crossed the limit imposed by the cell membrane, take the name of exosomes.

– Exosomes are miniaturised "fingerprints" of the cells that generated them.

– In addition to RNA, EXOs can also contain fractionated DNA (Sharma & Johnson, 2019; Spada *et* Al., 2020).

In addition to RNA, EXOs contain proteins such as the **4 tetraspanins** involved in the penetration of EXOs into recipient cells and fusion events, the **Heat Shock Proteins** (HSPs) involved in presentation, antigen binding and the formation of MVB (release of EXO), and **Cell Adhesion Proteins**.
The EXO cargo is also rich in lipids such as cholesterol, sphingomyeline, ceramides, phosphatidyl-serine, and arachidonic and phosphatidic acids.
– The typical composition of an EXO is shown in FIG. 5.

INTERCELLULAR COMMUNICATION

Cells normally communicate with neighbouring cells through direct contact (gap junctions, surface proteins), or remotely (hormones, chemokines, cytokines), or via electrical and chemical signals (nucleotides, short-chain peptides and lipids).
– EXOs have completely different, peculiar ways of communicating: from parent cell to recipient cells they penetrate directly into them, near or far. Once they have penetrated the recipient cells, they stimulate them using ligands located on their surface, transfer the activated receptors, and **epigenetically reprogramme** them by delivering their cargo.
A true... home delivery.
– This unique, emerging behaviour in Biology and Medicine accounts for, for example, their enormous importance in immunology, such as activation, suppression, immune tolerance, and antigen presentation (Raposo *et* Al., 1996; Zitvogel *et* Al., 1998; Clayton *et* Al., 2011; Zhang *et* Al., 2011; Smyth *et* Al., 2013; Okoye *et* Al., 2014); EXOs derived from mesenchymal stem cells have shown enormous potential in reducing the extent of myocardial infarction (Lai *et* Al., 2010; Liu *et* Al., 2017), in renal infarction (van Koppen *et* Al., 2012), in the healing of wounds (Zhang *et* Al., 2015), in liver disease (Tan *et* Al., 2014; Jiang *et* Al., 2018), in the protection of neurons (Xin *et* Al., 2012), in the reduction of retinal damage caused by laser surgeryr (Yu *et* Al., 2016), in addition to the control of viral pathogenicity, and the treatment of premature ageing, to name the just most striking examples.

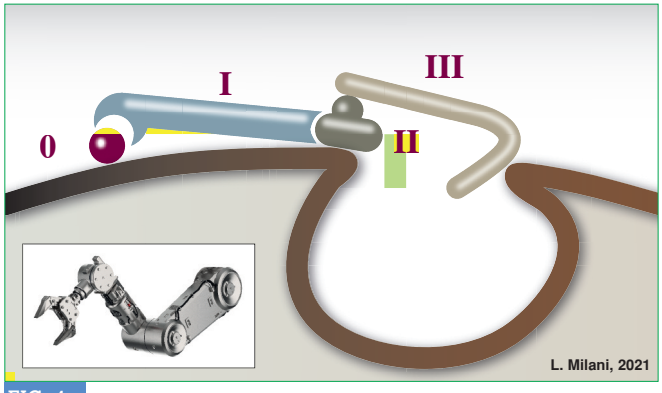


FIG. 4

Schematic graphics of the ESCRT (Endosomial Sorting Complex Requiring for Transport) machinery, made up of IV protein subunits in line (0, I, II, III), which "manufactures" the invaginations and "welds" the membranes from endosome to EXO.
Like a robotic arm, ESCRT-I pulls ESCRT-0 towards itself by "curling" the plasma membrane, while ESCRT-II pushes it down. When an endovesicle has formed, ESCRT-III approaches the 2 lipoprotein neck flaps of the parent membrane.
• ESCRT is an ancient phylogenetic mechanism. It also found in the Archaea that appeared on Earth 3.5 billion years ago, the prokaryotes that can survive in extreme environmental conditions.
Nature loves patterns and repeats them.

– Adapted from Raiborg & Stenmark, 2009 (see References).

EXOSOMES AND LIVER DISEASES

The therapeutic efficacy of EXOs from mesenchymal stromal stem cells (MSCs) and hepatocytes has been studied in animal models *in vitro* and *in vivo*.
Below is an updated and exhaustive list of the most significant bibliographic references, in ascending chronological order:

1. **HEPATIC FIBROSIS** - Masyuk *et* Al., 2013; Charrier *et* Al., 2014; Hyun *et* Al., 2015; Chen *et* Al., 2016; Nojima *et* Al., 2016; Lou *et* Al., 2017; Shen *et* Al., 2017; Qu *et* Al., 2017; Chen *et* Al., 2018 a; Mardpour *et* Al., 2019; Povero *et* Al., 2019; Rong *et* Al., 2019; Fiore *et* Al., 2020; Bruno *et* Al., 2020 a;b.

2. **HEPATIC CARCINOMA** - Kogure *et* Al., 2011; Fonsato *et* Al., 2012; Sun *et* Al., 2018; Li *et* Al., 2020.

3. **HEPATITIS, DRUG-INDUCED HEPATOTOXICITY** - Li *et* Al., 2013; Lemoinne *et* Al., 2014; Tan *et* Al., 2014; Abbas & Hamideh, 2014; Brigstock, 2015; Cai *et* Al., 2017; Chen *et* Al., 2018 b.

4. **NON-ALCOHOLIC STEATOHEPATITIS *(NASH)*** - Bruno *et* Al., 2020 b.

5. **LIVER REGENERATION AFTER PARTIAL HEPATECTOMY** - Herrera *et* Al., 2010.

6. ***BIOMARKER* OF LIVER DISEASES** - Wang *et* Al., 2017; Barile & Vassalli, 2017.

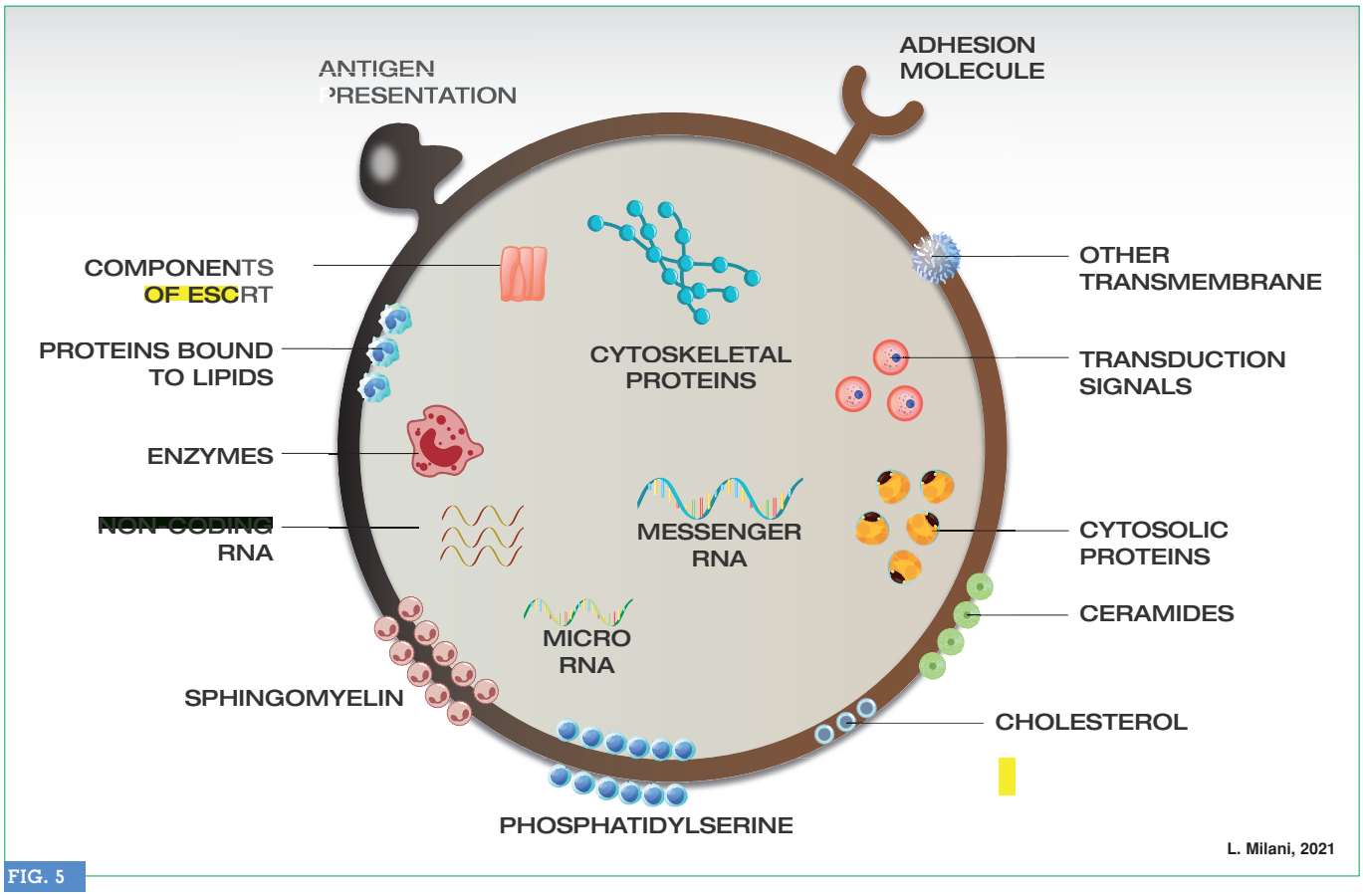


FIG. 5

Typical composition of an exosome. The cargo.

These data from the most recent scientific literature emphasise the major therapeutic impact offered by EXOs in the treatment of liver diseases, their antidegenerative, antispasmodic effects, and their potential use as biomarkers of liver diseases.

▶ In this context, we should mention **Epatoguna** (Guna Laboratories - Milan, Italy), a food supplement based on hepatic lyophilisate processed intact from healthy young swine according to the Neorland® method, choline and green tea.
– The hepatic lyophilisate in Epatoguna is a source of well-preserved exosomes that are biologically active on human stem cells, as can be clearly seen in the results of the experiment conducted by the research group coordinated by Prof. C. Ventura – CNR, Bologna, Italy (Ferroni *et* Al., 2019).

– I quote the authors (from the discussion in the article): 1) "*Neorland® swine's liver contains exosomes*; 2) *these are internalised by human cells and are biologically active*". Neorland® hepatic lyophilisate is found exclusively in Epatoguna.

– **Choline** is a lipotropic substance that prevents the deposition of lipid in the liver parenchyma.
Diets with a low choline content favour NAFLD (Non-Alcoholic Fatty Liver Disease), the current, more correct definition of the "old" hepatic steatosis.
The nutraceutical intake of choline boosts the synthesis of very low density lipoproteins (VLDL), thus facilitating the transport of triglycerides outside the cell and avoiding their accumulation in the cytoplasm of the hepatocytes (Lombardi *et* Al., 1968; Yasuda *et* Al., 2020).
– **Green tea** [(*Camellia sinensis* (L.) Kuntze] (titrated at 95% in epigallocatechin-3-gallate) protects the liver parenchyma and reduces inflammatory markers; it lowers hepatic triglycerides (Xu *et* Al., 2020), ALT (Yu *et* Al., 2017), and regulates insulin resistance (Pham *et* Al., 2014).

THE BOTTLE ON THE BEACH

The discovery that lymphocytes also have receptors for hypothalamic hormones (CRF, GHRH, LHRH), hormones (cortisol, prolactin,

GH, melatonin), neuroendocrine peptides (enkephalins, ß-endorphin, somatostatin, NGF, ACTH, substance P, CGRP), neurotransmitters (adrenaline, noradrenaline, acetylcholine, dopamine) which, in turn, release neuroendocrine peptides, and that immune function is also affected by some hormones, neurotransmitters and other messengers (in Milani, 2018) strongly suggests that there is a powerful **information exchange** between P, N, E and I.

Each one of the 4 "performers" in the PNEI superSystem is **only** effective if it is interconnected at the same time as the other 3, thus ensuring "true" psycho-organic homeostasis. None other.

– Each of these 4 "performers" acts remotely through electrochemical mechanisms, synapses, ligands, etc. or modifications of its own cells or those of the recipient, not necessarily target cells.

– Everything in Nature is information.
The Whole itself is information, in formation.

Networks and networks of networks are the guarantors of alignment of the expressions of Life.
They oversee the domains of coherence.

– A single hypo/hyper imbalance between them alerts the entire individual in a compensatory attempt to restart the jammed, highly complicated clock.

• A 5th has recently been added to these 4 Systems: the exosomal/microvesicular system which, through short and long range intercellular communication, transfers microRNA and messenger RNA that affect the recipient cells along their own "journey", carried in bodily fluids, reaching their destination as… beached messenger bottles - Neptune's treasure.

– Studies on EXOs have literally exploded exponentially in the last 5 years, opening up new, unexpected, fascinating opportunities in early diagnostics (biomarkers) (Simpson *et* Al., 2009; Heubner *et* Al., 2015; Fais *et* Al., 2016) and in therapy as drug delivery systems (El Andaloussi *et* Al., 2013; Johnsen *et* Al., 2014; Vader *et* Al., 2016; Batrakova *et* Al., 2016; Ingato *et* Al., 2016) or as EXOs processed from healthy **animal** or **plant tissue** that are biocompatible with human tissue.

– The therapeutic effect of stem cells is most likely mediated by the release of the EXOs they produce: these have the ability to recapitulate the effect of stem cells and – consequently – bring further life to their practical applications… regenerative medicine, the dream – now more and more tangible – of every doctor and every patient. ■

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