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Comparison of Two Endogenous Delivery Agents in Cancer Therapy: Exosomes and Ferritin

Le Li,[1] Lianbing Zhang,*[1] Mato Knez*[1,2]

[1]CIC nanoGUNE, Tolosa Hiribidea 76, 20018 Donostia-San Sebastian, Spain.

[2]IKERBASQUE, Basque Foundation for Science, Maria Diaz de Haro 3, 48013 Bilbao, Spain.

*Correspondence to: l.zhang@nanogune.eu; m.knez@nanogune.eu

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Abstract:

Exosomes and ferritin: Two biomacromolecules from our human bodies both draw increasing interest for advanced drug delivery due to their endogenous origin and their morphology, the cage-like structures. They possess perfect naturally designed structures for loading and shielding of cargo. Their intrinsic biological functions enable a natural delivery of the load and specific targeting. More and more evidences point towards the evolution of a new era of drug delivery strategies with exosomes and ferritin, even for potential personalized therapy. This review focuses on the advantages as well as limits of exosomes and ferritin as endogenous carriers for cancer therapy. We compare their structure-specific cargo loading and their intrinsic cancer-related biological functions. Remaining challenges and promising perspectives for future development to use these two endogenous agents are discussed.

Introduction

As an invasive disease cancer is one of the major causes for human death worldwide and the number of cases keeps increasing every year [1]. Efficient delivery of therapeutic agents and the specificity to target cancerous tissue are the main pillars for successful clinical therapies, especially chemotherapy. In case those are not fulfilled, severe side effects and toxicity will limit the use and efficiency of the therapeutic agents in clinical practice [2-4]. The clinical outcome of the various products for gene-therapy is even more unsatisfactory [5-6]. Here, the major obstacles include rapid extra- and intracellular enzymatic degradation and low cellular uptake. In addition, the nuclear membrane represents an additional barrier for successful drug delivery, especially when DNA-based products are involved.

Many delivery systems have been developed to overcome the barriers. Requirements for a successful delivery system include safety (biocompatibility and biodegradability), capability of entering the appropriate cellular compartment, and specificity towards the target sites. Viral delivery agents have been used most successfully in the field of gene therapy because of their high transfection efficiency. However, the inability of viral vectors for loading a variety of drugs, induction of unwanted immune responses and unpredictable cytotoxicity (for example, tragic death of a patient receiving an adenoviral vector) remain critical for clinical applications of viral vectors [7-10]. In order to increase the delivery efficiency, intensive investigations show a promising potential of nanotechnologies in human medicine. In recent years, various approaches and different techniques have been attempted for delivery of drugs in a reduced dose, with specific targets and controllable release,

including applications of nanomaterials with inorganic, synthetic polymeric, biological or hybrid composition [11-13]. However, all these non-viral delivery agents face the same hindrances and biological barriers on the way of delivery: The unsatisfactory delivery efficiency and toxicity due to the synthetic nature limit the clinical use of the most of the synthetic non-viral agents [14, 15].

In order to avoid the obstacles imposed by delivery vehicles composed of exogenous materials (both viral and synthetic agents), exploring endogenous cellular components for delivery lately became an interesting concept [16-18]. Biomolecules that are involved in cellular uptake pathways are of particular interest, since they can easily pass cellular barriers making use of the natural cellular machinery. For example, various cell-penetrating peptides, receptor-specific ligands and endosome-escape fusiogenic peptides have been explored as delivery agents [18]. Still, most of them cannot carry drugs by themselves (except for few that can interact and deliver nucleic acids) and are employed as modification motifs for actual cargo carriers. Thus, two of such biomolecules emerged to become the most popular investigation subjects in this new concept: exosomes and (apo)ferritin [16-21]. The reasons lay in their different but unique structural and biological features. Although exosomes and (apo)ferritin have completely different sizes and composition, they both possess natural hollow spherical structures as potential container for cargo loading without further engineering. Their intrinsic biological roles in the intercellular communication (exsome) or intracellular storage of the essential mineral (ferritin) define their distinct cellular uptake and intracellular distribution, which favors their use for cargo delivery. The fact that both exosome and ferritin already play important roles in progress of cancer, this makes their applications in cancer therapy extremely interesting [22,23].

In this mini review we emphasize especially the advantages provided by their distinct biological functions. Their merits and limitations will be compared. A discussion of the remaining challenges will attempt to reveal their still unexploited cancer-related functions that are of interesting potential for their use and further development as drug delivery agents in future. For extended and comprehensive reviews of exosomes and ferritin in biology, cancer therapy and nanotechnology the reader may refer to excellent reviews recently published elsewhere [24-29, 30 this issue].

1. Structures and composition

As soon as someone compares exosomes with ferritin, the first obvious differences are clearly found in their composition and size (Fig.1). In general, the structure and the composition of ferritin are precisely defined, while the lipid bilayer structure of exosomes is much larger in size and the composition is more complex.

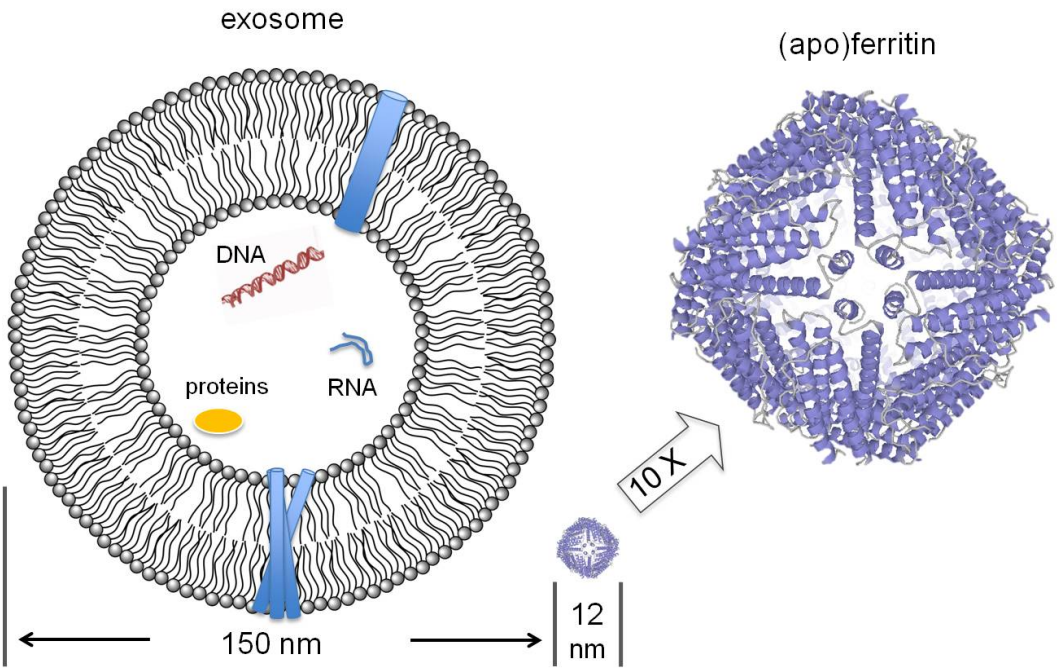

Figure 1. Schematic of the size and composition of an exosome and (apo)ferritin.

Exosomes

Exosomes are small vesicles formed by a lipid bilayer membrane. They have been defined as originating from multivesicular bodies (MVBs) and are secreted upon fusion of MVBs with the plasma membrane [31]. Together with shedding microvesicles and apoptotic bodies, they form the three main classes of extracellular vesicles (EVs) that are released constitutively or upon activation by almost all cell types, including tumor cells [32–35]. EVs carry a broad range of cargoes, including proteins (cytokines, membrane receptors, and receptor ligands, etc.) and nucleic acids (DNA, mRNA, long and short noncoding RNA, etc.) [21].

The size of exosomes ranges from 40 to 150 nm with a buoyant density of 1.13–1.19 g/cm3 [36]. In comparison, shedding microvesicles are formed through direct outward budding of the plasma membrane and apoptotic bodies are released upon fragmentation of cells undergoing apoptosis. The sizes of microvesicles and apoptotic bodies are more heterogeneous and vary between 50 and 5000 nm [21]. Both exosomes and microvesicles are in normal physiological conditions important for the intercellular transfer of lipids, RNA, and cytosolic proteins. Each of three EVs has a different cellular generation pathway and fulfills distinct functions [25]. However, current isolation and detection techniques do not allow for a clear distinction of these three vesicular subpopulations, thus in many published studies “exosome” was generally used for all isolated EVs.

Ferritin

Comparing with exosomes, ferritin is much smaller and exactly defined in size. Its composition is much simpler. As one of first proteins to be identified and named, ferritin is a ubiquitous and major iron storage protein in all organisms with the

exception of yeast and centric diatoms [27]. The overall structure of eukaryotic ferritin is almost identical with an iron mineral core surrounded by a protein cage. The protein cage without iron mineral is called apoferritin, which is an almost spherical shell of 12 nm composed of 24 subunits and defines a cavity of 8 nm in diameter for iron storage [27, 37].

The ferritins from human and some other eukaryotes are generated by self-assembly of two subunit types: the heavy (H)- and light (L)-chains [27, 37]. The H-chain has the ferroxidase center and is responsible for the oxidation of Fe^{2+} to Fe^{3+} and the L-chain, without ferroxidase activity, facilities the iron nucleation and the electron transfer across the protein shell [27,38,39]. Two types of channels perforate the protein cage: hydrophobic channels on the 4-fold axes and hydrophilic channels on the 3-fold axes [27]. The hydrophilic channels are lined by carboxy groups and serve as the passageway for ions, including iron and a variety of metal ions [40,41]. Recently, the pockets at the 2-fold intersubunit of mammalian ferritins are found to be able to bind organic molecules, such as fatty acids [42].

2. Cargo loading

The structural and compositional differences of exosomes and ferritin lead to different methods for the cargo loading. Cargoes have to pass the lipid bilayer of exosomes or the protein cage of ferritin in order to enter into the lumen of the cavity. The type of cargos that can be loaded or encapsulated into exosomes and ferritin is not exactly the same.

Exosomes

The basic structure and composition of the exosomal lipid membrane is quite similar to that of a cell membrane or engineered liposomes. The methods and techniques developed for effectively loading liposomes or transfecting living cells are in principle applicable to isolated exosomes as well.

The simplest method for loading is the direct incubation of exosomes with the cargo. Some small molecules, for example, miR-150 and doxorubicin, can be loaded into the lumen through diffusion across the lipid bilayer of the exosome [43,44]. This simple method works extremely efficiently with some hydrophobic molecules that can cause lipid rearrangement and change the lipid fluidity [45]. For example, an efficient loading of curcumin into exosomes was achieved after only 5 min of incubation at 22 °C [46].

For large and charged molecules that cannot diffuse across the lipid membrane, loading can be achieved with electroporation or assisted with chemical transfection reagents like Lipofectamine [47-49]. siRNA was successfully loaded into exosomes with Lipofectamine 2000. However, it is difficult to remove the excess micelles formed by siRNA and lipofectamine from the exosomes after loading. Therefore, this approach has the risk of introducing contamination from synthetic polymers and is an inadequate loading method for therapeutic purposes [48,49].

With electroporation an electrical field is applied that creates pores in the lipid bilayer membrane of exosomes, thereby facilitating the entry of the cargo into the lumen of the exosomes [50]. Loading of siRNA into exosomes via electroporation was successfully performed in several attempts [47-49]. Chemotherapeutics like doxorubicin, which could be loaded with direct incubation, were also loaded with electroporation [51]. However, loading exosomes with electroporation is not always straightforward. For example, Kooijmanns et al. recently demonstrated that siRNA

may aggregate during the process of electroporation, which greatly influences the siRNA loading efficiency [52]. Since electroporation can also induce aggregation of exosomes, in order to achieve desired loading efficiency, the optimal parameters (voltage, concentration of exosomes and cargo, buffer, etc.) may vary for different cargos and with exosomes isolated from different donor cells.

Inspired by the natural function of the exosome for intercellular transport of RNA and proteins, one of the currently most appropriate and widely used method for exosome loading is transfecting the exosome-producing cells to overexpress the desired gene products that will be packed into the exosomes (lumen or membrane). For example, proteins, shRNAs or miRNAs can be loaded into exosomes by transfection of donor cells with cargo-expression vectors or pre-miRNAs [53-57]. In one of these studies, miR-146b loaded exosomes were produced by mesenchymal stem cells (MSCs) after transfection with a miR-146b expression vector and the resulting exosomes could inhibit the cancer growth [53]. Direct incubation of drugs with exosome-producing cells may also lead to the loading of exosomes with drugs. For example, exosomes were loaded with the anti-cancer drug paclitaxel in this way [58]. The requirements to load the drug in this way are a high exosome-producing ability of the donor cells and a low toxicity of the drug to be loaded for the donor cells.

The transfection of donor cell is not only an approach for loading of therapeutic cargo into exosomes but also a very effective bioengineering technique to modify the surface of exosomes in order to achieve better delivery targeting and efficiency [51,53,54]. One interesting case is the production of exosomes with overexpressed MHC-II (major histocompatibility complex class II molecules) on the surface, which enables the targeting towards T-cells and also the provocation of immune responses of T-cells against cancer cells [56]. In this case the transfection of the donor cells to

overexpress MHC-II fulfills the cargo loading and targeting modification spontaneously. Other targeting peptides or proteins used to modify exosomes through the donor cell transfection include folate receptor α, RVG, EGF, iRGD, etc [47,51,54,59].

Ferritin

Eight hydrophilic and six hydrophobic channels perforate the ferritin protein shell [40,41]. The hydrophilic channels serve as the transport pathway of iron ions upon natural mineralization and demineralization of ferritin. The diameter of these small channels is in the Ångstrom range, large enough for several small therapeutic molecules to pass through. Therefore, direct incubation with the cargo is also an applicable loading method for ferritin, or more precisely, for apoferritin [60-62]. For example, the Cu^{2+}-doxorubicin complex could be successfully loaded into the cavity upon direct incubation with the recombinant human H-chain apoferritin [60]. Hydrophobic drugs were also loaded into the cavity through the hydrophobic channels. For example, Gefitinib, an EGFR tyrosine kinase inhibitor, was loaded into recombinant human apoferritin through the hydrophobic channels [62]. Generally, this approach was frequently used to load smaller reactive precursor molecules into the cavity in order to obtain the final cargos with a larger size through subsequent reactions. In this way the cargo is synthesized in the cavity and cannot diffuse outwards because of the too small channels [61,63]. The anti-cancer drug, cisplatin, was synthesized inside the cavity by subsequent incubation of apoferritin with K_2PtCl_4 and ammonia-ammonium chloride [63]. A modified approach enabling the loading of slightly larger cargos is through widening the channels by partially denaturing the channel with detergents. Subsequent removal of detergents will restore

the channel structure, which will trap the cargo inside the apoferritin cavity [64,65]. The process of denaturation of proteins with 8 M urea and renaturation with gradual urea removal was recently used to encapsulate doxorubicin, carbachol and atropine into apoferritin [66].

A major benefit of ferritin is that its subunits can be disassembled and re-assembled to form the 24-mer shell again. In mammalian cells the H- and L-chains assemble in the proportion specific for each organ or tissue [67]. In vitro the disassembly of apoferritin can be simply achieved by change the pH [68]. The reassembly of the shell occurs spontaneously when the pH is restored. The self-assembly process is extremely effective and after the reassembly nearly no free subunits are left. There is evidence that the formation of hetero- over homo-polymers is strongly preferred, which allows the production of heteropolymeric apoferritin with desired H/L proportion [39,69]. The easy formation of assembled/disassembled structures of ferritin breaks the restriction of the channel size and expands the spectrum of therapeutic cargos for loading the apoferritin cavity up to its size limit. Although the mechanism is not totally understood, this approach is the common method for loading apoferritin [63, 70-73]. Various drugs and materials for potential cancer therapy were successfully loaded, including doxorubicin, cisplatin, nanoparticles and imaging agents etc. Ferritin, as a protein-based delivery agent, can also be easily genetically modified. However, modifications of ferritin with bioengineering techniques are mostly applied with the aim to improve its targeting selectivity [60, 73].

Since apoferritin is much more stable and easier to handle than exosomes, the cargo loading into apoferritin is easier and more straightforward than into exosomes. The amount of loaded cargo can be controlled to some extent simply by adjusting the incubation time or the drug/apoferritin ratio. With exosomes, a similar control of

loading dosage is only possible upon direct incubation. Since the mechanism of cargo sorting into exosomes is not fully understood [74], cargo loading by donor-cell transfection still remains being a random and uncontrolled process at the current stage. However, due to the volume limit of the cavity, the loading capacity of apoferritin, regarding to the cargo quantity and diversity, is more restricted if compared with exosomes.

3. Important biological functions for cancer therapy

Exosomes and (apo)ferritin are often referred as "smart" delivery agents due to their natural tailored biological properties that provide unique advantages in delivery. These useful properties include their intrinsic involvement in cancer by themselves, their self-triggered cellular uptake and their distinct subcellular distribution.

3.1 Cancer Biomarkers

Exosomes

It is increasingly accepted that the exosome-mediated intercellular communication plays an important role in tumor development and progression, including immune suppression, angiogenesis, and metastasis [75-77]. Since exosomes carry the unique fingerprint of cells from which they are derived, an analysis of this signature (size, cargo and composition) will help to understand the exact functions of exosomes in cancer and to target exosomes as a therapeutic avenue [21]. In addition, the knowledge of interaction mechanisms of exosomes from different origins with recipient cells will improve the use of exosomes as drug carriers. For example, it has been found the intratumorally injected tumor-derived exosomes (mouse mammary carcinoma 4T1 cells), but not the engineered liposomes, can strongly associate with a

tumor tissue (Fig. 2) [78]. However, the intravenously injected unmodified tumor-derived exosomes, as well as liposomes, showed insufficient enrichment in tumor tissue (Fig. 2).

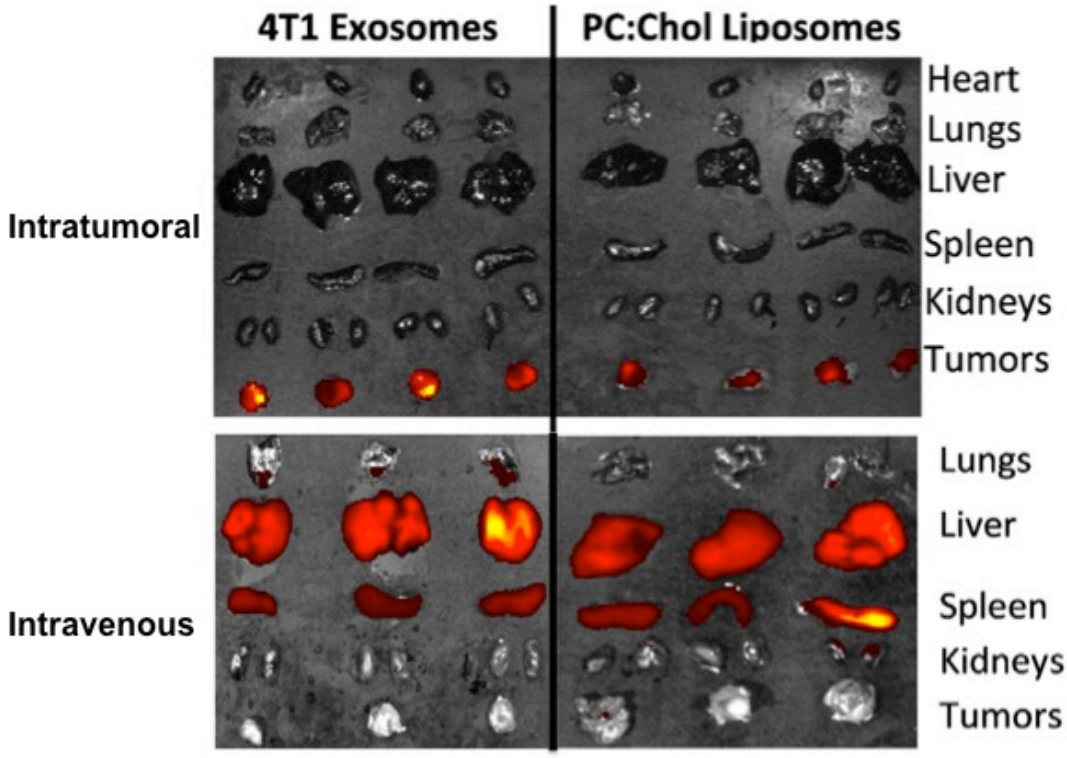

Figure 2. The fluorescence intensity of organs from mice receiving intratumoraly and intravenously either tumor-derived exosomes (mouse mammary carcinoma 4T1 cells) or liposomes after 24h. Injected exosomes and liposomes were labeled with equal amount of fluorescence dye. Reprinted with permission from ref. [78]. Copyright 2015 Elsevier.

Ferritin

Increasing evidence suggests key roles of ferritin, especially serum ferritin, in iron delivery, proliferation, angiogenesis and immunosuppression of cancer [23]. Intracellular ferritin is overexpressed in several tumors, such as hepatocellular carcinoma, breast cancer and pancreatic cancer [79-81]. Also the level of extracellular

ferritin (serum ferritin) is elevated in patients with those cancers [79,82-84]. Similar to exosomes, the involvements of ferritin in cancer make it becoming one of the promising therapeutic targets for cancer treatment. A siRNA-mediated knockdown of H-chain ferritin sensitized glioma cells to chemotherapeutic agents [85].

3.2 Cellular Uptake

Undefined or insufficient cellular uptake is one of the shortcomings of synthetic delivery agents. Tedious surface modifications are often required for improvements. In contrast, the role of exosomes in the intercellular communication and existence of ferritin receptors designate the presence of inherent pathways for their cellular uptake. Natural cellular uptakes provide possibilities to use them as "Trojan horses" for drug delivery in the fight with cancer diseases.

Exosomes

The mechanism of cellular uptake of exosomes is not clearly understood. The current discussion is focused on whether this occurs through endocytosis/phagocytosis or direct membrane fusion [25]. Whatever the mechanism is, exosomes can obviously interact with recipient cells via various mechanisms. Membrane proteins on the surface of exosomes are key components for the cellular uptake via direct interactions with receptors on the target cell. This leads to a higher uptake efficiency and target cell selectivity, which is difficult to achieve with synthetic liposomes, even with the same lipid composition as in exosomes [86]. It has been shown that the exosomal tetraspanin web contributed to the target cell selectivity [87]. However, the quick clearance of intravenously injected exosomes has been observed in several in vivo

studies, which suggests a very limited ability of unmodified exosomes for targeted drug delivery [51,78,88].

Ferritin receptors

The cellular uptake of ferritin has been known for a long time to occur through endocytosis (Fig.3) [89]. In 2005, the mouse TIM-2 was identified as the H-chain ferritin receptor based on the observation that TIM-2 is expressed on various cells and binds H-chain, but not L-chain ferritin [90]. However, the TIM-2 homologue has not been found in humans. The major human ferritin receptor was found in 2010 to be the transferrin receptor-1 (TfR1) that also has a preference towards H-chain ferritin [91]. Since TfR1 is ubiquitously expressed in almost all cells for their essential iron uptake, the majority of the studies until now are focused on using H-chain apoferritin as the delivery agent [61,62,65,71,72,92]. Actually, Scara5, a receptor expressed in mouse and human, is able to bind and take up L-chain ferritin [93]. However, no study has been carried out to yet make use of this L-chain specific pathway for drug delivery.

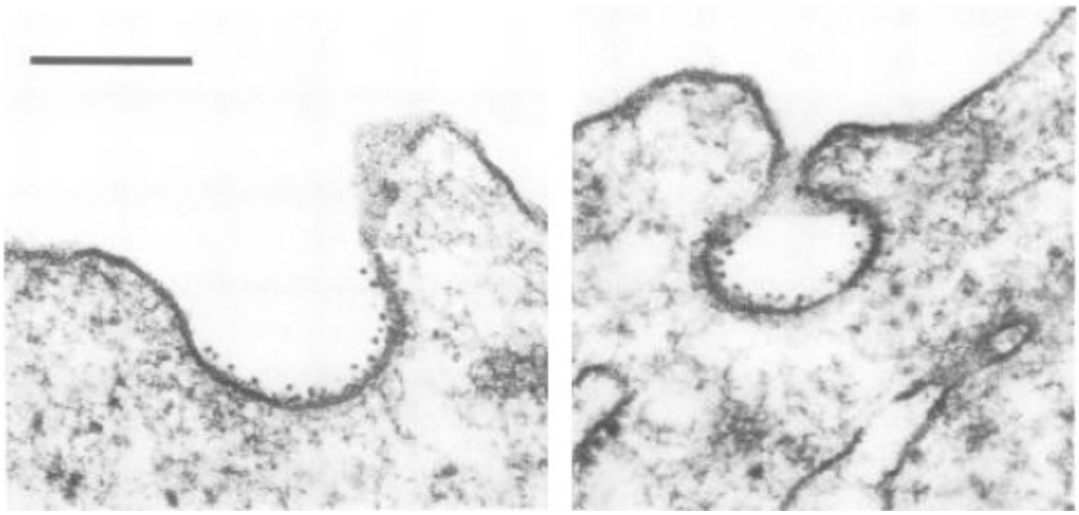

Figure 3. The binding and uptake of ferritin by giant HeLa cells through endocytosis. Scale bar: 0.2 μm. Reprinted with permission from ref. [89]. Copyright 1983 Macmillan Publishers Ltd: the EMBO Journal.

3.3 Nuclear Ferritin

The location of ferritin in cell nuclei was first observed under pathological conditions in cells such as hepatocytes of mice following an iron overload [94]. Nuclear ferritin in cells under normal physiological conditions was later detected in chicken corneal epithelial cells and in rat neurons [95, 96]. Studies also showed a translocation of H-chain ferritin, but not L-ferritin, from the cytosol into the nucleus. The translocation is independent of the ferroxidase activity [97]. A later distribution analysis of rat tissues demonstrated a differentiation of H- and L-chain ferritin: strong cytoplasmic and very weak nuclear expression of L-chain vs. strong nuclear and weak cytoplasmic localization of H-chain in liver, spleen, heart and brain [98].

The ability of nuclear translocation of H-chain ferritin brings further advantages for its use as delivery agent, since the target of many anti-cancer drugs is DNA, located in the nucleus. Recent studies from two groups demonstrated a rapid nuclear delivery of doxorubicin with recombinant human H-chain ferritin (Fig. 4a)[71,72]. Due to the rapid delivery to its target site, the delivery via nuclear translocation bypassed the multidrug resistance and elicited the therapeutic potential of the drug (Fig. 4b) [71,72]. Combined with its receptor-mediated uptake, H-chain ferritin emerged to be a natural carrier capable of passing two cellular barriers without further modification.

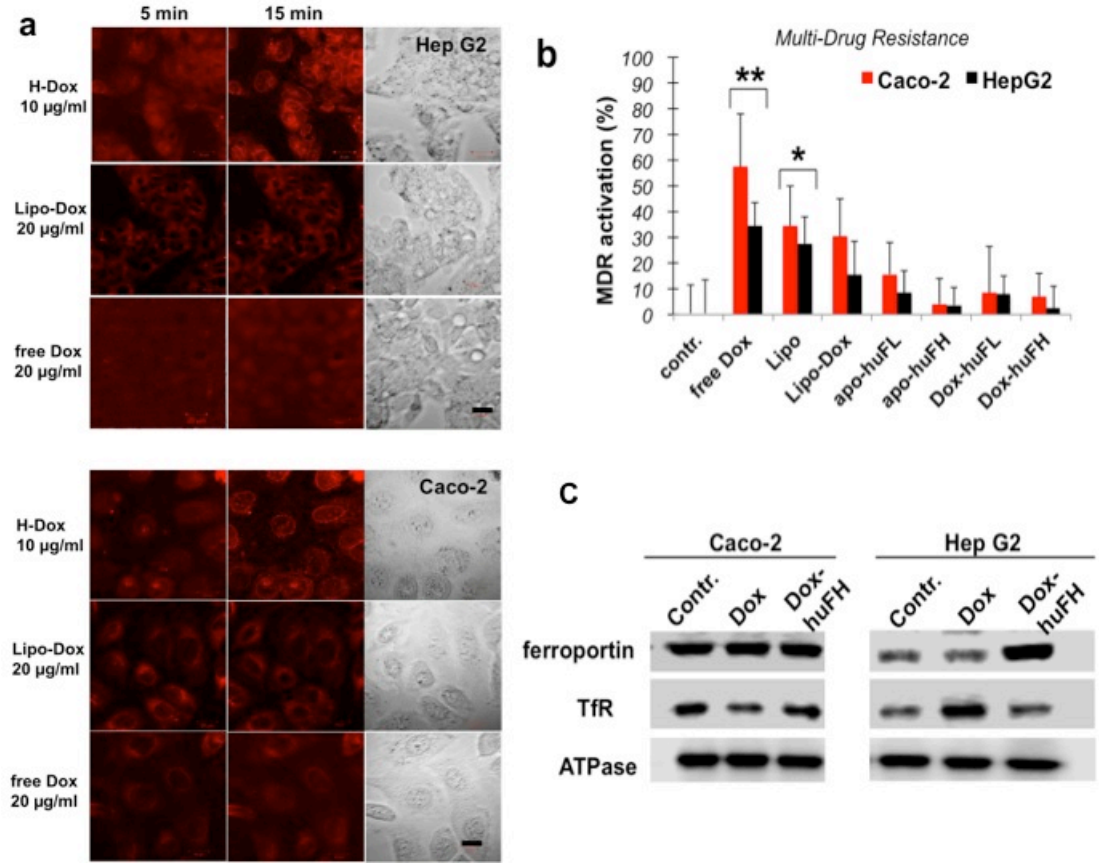

Figure 4. a) Quick nuclear delivery of Dox in HepG2 and Caco-2 cells with human H-apoferritin (H-Dox) compared with 20 μg/mL free Dox and liposomal Dox (Lipo-Dox). Scale bar: 20 μm. b) Cellular multidrug resistance level of Caco-2 and HepG2 cells after treatment with Dox either as free Dox or encapsulated in liposome or ferritin for 24 h. The multidrug resistance activity of untreated cells was used as control (Contr.). c) Western blot detection of the transferrin receptor (TfR) and ferroportin in the cell membrane fractions of Caco-2 and HepG2 cells. Na^+/K^+ ATPase served as the loading control for the membrane fractions. Reprinted with permission from ref. [72]. Copyright 2015 Wiley-VCH Verlag GmbH & Co. KGaA.

4. Challenges and Perspectives

Exosomes

The production of exosomes for real clinical use is the greatest challenge [24,25]. Exosomes exist and can be isolated from cell culture medium and several types of

extracellular fluids including blood, urine, amniotic fluid, saliva, and cerebrospinal fluid [99]. Solvent precipitation, column chromatography, ultracentrifugation and immunoaffinity methods are availed for exosome isolation [99,100]. However, as mentioned before, current isolation and detection techniques do not allow for a clear distinction of exosomes from other vesicular subpopulations. Furthermore, studies demonstrate that different isolation methods have different effects on the yield and physiochemical properties of the isolated exosomes [100,101]. Thus, the currently available isolation protocols need to be improved and standardized in order to increase the yield and the purity of the resulting sample.

For autologous exosomes to meet the requirements of clinical therapy, careful choice of the donor cell type is extremely important in order to devoid of any healthy risk. The involvement of exosomes in cancer development and progression encourages the idea of using exosomes as drug delivery vehicles. However, many proteins and RNAs found in tumor-derived exosomes are known for their roles in angiogenesis or inflammation, which support the tumor growth and spread [21]. For example, exosomes derived from activated platelets stimulated proliferation and invasion of lung cancer cells [102]. Exosomes released by tumor cells under hypoxia conditions also significantly enhanced the tumor growth [103]. Tumor-derived exosomes can even transfer P-glycoprotein, one of the membrane transporters responsible for multidrug-resistance, from resistant cells to drug-sensitive ones [104]. Even exosomes from non-tumor cells are not definitely safe. MSCs were frequently used as exosome donor cells because of the relatively high exosome production [53,58,105]. However, studies showed the cancer-stimulating properties of MSCs and MSC exosomes [106, 107].

By now, only little attention has been put to this issue and, as far as we know, no investigation has ever been conducted to investigate the effects of the inherent content in isolated exosomes on the therapeutic effects. The fact that more than 11,000 proteins (not to mention other biomolecules) have been identified in association with exosomes makes it impossible to deplete any of them before identifying the exact exosomal function [108]. It could evolve to a double-edged sword, since the components with cancer-stimulating effects may be also indispensible for exosomal targeting or cellular uptake.

Another roundabout strategy is to use non-autologous exosomes or to fabricate exosome-mimetics from bacterial or human cells. Initial studies of exosome-mimetics show promising results [109-111]. However, further investigations are required to evaluate their clinical relevance. One clinical trial in phase I stage is currently investigating plant-derived exosomes for oral curcumin delivery for colon cancer treatment (NCT01294072). Since synthetic liposomes have been investigated since more than 30 years for drug delivery in cancer therapy, it cannot be denied that their reproducibility and therapeutic effects are more significant than those of exosomes in the current stage. However, intensive ongoing investigations on exosomes and the accumulating knowledge of the exosomal composition for their biofunctions brings also opportunities to use the natural exosomal design as the blueprint for a further development of synthetic liposomes for cancer therapy.

Ferritin

The possibility to produce human (apo)ferritin with recombinant techniques solves the problem of its isolation difficulties from tissues and enables the potential application of apoferritin in a large clinical scale. Several studies have already

demonstrated the low toxicity of recombinant ferritin in vitro and in vivo [60-63,71,72,112]. However, due to the volume limit of the cavity, the loading capacity (drug amount and diversity) of apoferritin is more restricted than it is the case with exosomes. The inability of apoferritin to encapsulate genetic materials with large sizes (e.g. plasmids) makes the application of (apo)ferritin in gene therapy extraordinarily rare. One single study showed successful delivery of siRNA to cancer cells with (apo)ferritin by binding the siRNA to the outer surface of the protein shell [113]. However, it is unclear whether or not the occupation of the outer surfaces will disturb the binding of ferritin with its receptors, which will be a serious drawback for the cellular uptake. Another open question is to which extent it will impact the nuclear translocation of ferritin, which is actually a great advantage for gene delivery. (Apo)ferritin undoubtedly has a rather rigid structure, which is in contrast with the need of flexibility for drug loading. Whether the structure can be further optimized and engineered remains unpredictable and a success in this field will be of great interest for technical applications of ferritin.

In similarity to exosomes, natural involvements of ferritin in cancer include the immunosuppression, angiogenesis and proliferation stimulation [23]. For example, it has been shown that ferritin binds to cleaved high-molecularmass kininogen (HKa), an endogenous inhibitor of angiogenesis, with a high affinity and eliminated the anti-angiogenic effects of HKa [114]. Ferritin opposed also in vivo HKa's anti-angiogenic effects in a human prostate cancer xenograft and restored tumor-dependent vessel growth [115]. Therefore, caution must be taken upon use of (apo)ferritin as delivery agent in cancer therapy. It has to be evaluated whether the exogenously administrated drug-ferritin composite affects cancer also in a supportive manner.

Sophisticated combination of the biological function of ferritin with the delivered drug may achieve a maximal use of the ferritin. The major and fundamental function of ferritin is to oxidize and incorporate iron and keep it in a non-toxic form. The function of ferritin in iron homeostasis can have a large number of further effects [116]. A cardioprotective role of ferritin has been confirmed by several studies. It has been demonstrated that metformin- and doxorubicin-induced expression of endogenous ferritin protected adult mouse and embryonic rat cardiomyocytes against doxorubicin toxicity [117,118]. Recently, in Caco-2 and HepG2 cells it was demonstrated that recombinant H-chain apofferitin, as the doxorubicin delivery agent, could also attenuate the doxorubicin-induced deregulations of TfR and ferroportin (Fig. 4c)[72]. However, clinical assessments are needed to confirm the potential benefits of ferritin for cadioprotection along with an efficient doxorubicin delivery. In addition, the immunosuppressive effects of ferritin seem to be specific to H-ferritin with an active ferroxidase center [119]. It may be interesting to test whether a delivery with mutant H-chain ferritin without ferroxidase activity will lead to better therapeutic effects without immumosuppression.

Ferritin is an object of active and intense investigations. The new insights into the molecule and its diverse functions will definitely bring fresh inspirations for its applications in cancer therapy. For example, besides the cytosolic and serum ferritin, mammals have a further type of ferritin located in the mitochondria [120]. Recently, a novel functional ferritin has been identified in humans and mice, named ferritin-heavypolypeptide-like-17 (FTHL17), which also assembles into the shell structure and partially accumulates in the nucleus [121]. These types of ferritins have not yet been considered for drug delivery and therapy.

It is known that different cancer patients may have different responses to the same drug, even if supplied in the same formulation [122-124]. Since exosomes and ferritin ubiquitously exist in everyone's body, it is of great interest and benefit having the possibility to extract or isolate them as autologous delivery agents. This feature may pave the way for fabricating personalized drugs for cancer therapy with delivery agents originating from the patients themselves. However, better understanding of the various diseases themselves and the biological functions of exosomes and ferritin are essential to realize this ambitious goal.

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