Exosomes Communicate Protective Messages during Oxidative Stress; Possible Role of Exosomal Shuttle RNA

Maria Eldh¹, Karin Ekström¹, Hadi Valadi², Margareta Sjöstrand¹, Bob Olsson³, Margareta Jernås³, Jan Lötvall¹*  
¹Krefting Research Centre, Dept. of Internal Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, ²Department of Rheumatology and Inflammation Research, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, ³Department of Internal Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

Abstract

Background: Exosomes are small extracellular nanovesicles of endocytic origin that mediate different signals between cells, by surface interactions and by shuttling functional RNA from one cell to another. Exosomes are released by many cells including mast cells, dendritic cells, macrophages, epithelial cells and tumour cells. Exosomes differ compared to their donor cells, not only in size, but also in their RNA, protein and lipid composition.

Methodology/Principal Findings: In this study, we show that exosomes, released by mouse mast cells exposed to oxidative stress, differ in their mRNA content. Also, we show that these exosomes can influence the response of other cells to oxidative stress by providing recipient cells with a resistance against oxidative stress, observed as an attenuated loss of cell viability. Furthermore, Affymetrix microarray analysis revealed that the exosomal mRNA content not only differs between exosomes and donor cells, but also between exosomes derived from cells grown under different conditions; oxidative stress and normal conditions. Finally, we also show that exposure to UV-light affects the biological functions associated with exosomes released under oxidative stress.

Conclusions/Significance: These results argue that the exosomal shuttle of RNA is involved in cell-to-cell communication, by influencing the response of recipient cells to an external stress stimulus.

Introduction

Exosomes are 30-100 nm extracellular membrane vesicles of endocytic origin [1-3], which were first discovered in the early 1980’s [1,4-5]. Exosomes are released into the extracellular environment upon fusion of multivesicular bodies with the plasma membrane [1–2,6]. They are secreted by most cells that have been examined so far, including mast cells [7–8], dendritic cells [9–10], B cells [6], T cells [11], tumour cells [12–13] and epithelial cells [14]. They have also been found in many biological fluids including plasma [13], urine [16], saliva [17], breast milk [18] and bronchoalveolar lavage fluid [19]. Exosomes were shown in the late 1980’s to have co-stimulatory functions in the immune system [6]. Furthermore, it has been shown that the exosome protein composition depends on the cellular source of the studied exosome [10,20]. Regardless of origin, several common proteins are found in exosomes, including chaperones, cytoskeletal proteins and tetraspanins such as CD9, CD63 and CD81 [3,8,20]. We have previously shown that exosomes also contain a substantial amount of RNA that can be transferred from one cell to another [8]. The functions of exosomes are not yet fully understood, although antigen presentation [6,21], induction of tolerance [22] and the transfer of genetic material [8] are the main proposed functions. The detailed mechanism of the interaction between exosomes and recipient cells are not fully understood, although experimentally supported hypotheses includes receptor-ligand interaction [6,21], fusion with the plasma membrane [23] or internalization of the exosomes by the recipient cells by endocytosis [24–25] followed by uptake of functional RNA [8]. Reactive oxygen species (ROS), including hydrogen peroxide (H₂O₂), are continuously generated during cellular metabolism in cells living under aerobic conditions. If the ROS production exceeds the production of the cells antioxidant defence, an imbalance occurs resulting in oxidative stress, which is implicated in many diseases including cardiovascular disease [26], sleep apnoea [27], asthma [28–29] and COPD [28]. In higher doses, H₂O₂ is capable of inducing oxidative stress in experimental models [30–31], which can lead to different types of cell death.
In addition, low doses of H\(_2\)O\(_2\) can induce tolerance of cells to a higher degree of oxidative stress [34–36]. Protection from oxidative stress has been shown to be regulated at the transcriptional level [37–39].

Since exosomes are produced and released by many cells, and have diverse functions in biological models [3,40], we hypothesized that exosomes may mediate protective signals in processes of oxidative stress. Thus, we suggest that exosomes released by cells exposed to oxidative stress can mediate a signal to another cell, making the recipient cell more tolerant to oxidative processes and subsequent cell death. We further hypothesized that any tolerising effect can be mediated by the exosomal shuttle of RNA, as we have previously shown that exosomes can deliver functional RNA from one cell to another [8]. To test these hypotheses, we used a mouse mast cell line (MC/9) that we exposed to H\(_2\)O\(_2\), as a model of oxidative stress.

**Results**

**Exosomes alter the ability of cells to handle oxidative stress**

It is known that oxidative stress induced by H\(_2\)O\(_2\) induces loss of cell viability in vitro [33]. Depending on cell type, the dose of H\(_2\)O\(_2\) needed to induce loss of viability differs. A dose-response evaluation was performed, after which we concluded that the concentration of 125 μM was optimal for our protocol as this dose caused the death of about 50% of the cells (Figure 1). It has previously been documented that cells pre-treated with a low H\(_2\)O\(_2\) dose develop a resistance to higher doses of H\(_2\)O\(_2\) and consequently to stress [34–36]. To determine whether exosomes released under oxidative stress can mediate a similar tolerising effect, we harvested exosomes from MC/9 cells exposed to H\(_2\)O\(_2\) or vehicle for 24 h. These exosomes were then added to untreated cultures of other MC/9 cells for 3 h, after which the recipient cells were exposed to oxidative stress at the same concentration. Recipient cell viability was examined at 0, 2, 12 and 24 h after H\(_2\)O\(_2\) exposure, by trypan blue dye exclusion. Cells pre-treated with exosomes harvested from conditions of oxidative stress, were shown to have a higher viability at the 0, 2, 12 and 24 h time points, compared to cells pre-treated with exosomes harvested from normal conditions (Figure 2).

**Exposure of cells to oxidative stress increase the relative amount of oxidized proteins in cells, but not in exosomes**

After showing that exosomes harvested from cells cultured under oxidative stress were capable of mediating resistance to oxidative stress, we next compared the degree of oxidization of cellular and exosomal proteins. This was performed by studying the carbonyl groups, introduced by the H\(_2\)O\(_2\) exposure, using a protein oxidation detection kit with a specific antibody targeting these carbonyl groups. We could, as previously shown [41], see an increase of oxidized proteins in cells exposed to H\(_2\)O\(_2\) (Figure 3a). However, the proteins in exosomes derived from cells exposed to H\(_2\)O\(_2\) did not express any change in the degree of oxidization (Figure 3b).

**Microarray analysis reveals that exosomes from different conditions contain different mRNA expression**

In our previous publication, we showed that exosomes contain not only protein, but also mRNA and microRNA [8]. Importantly, we also showed that the mRNA is functional and can be shuttled between cells. As we have shown that exosomes harvested from oxidative stress conditions affect the recipient cells extensively, we examined whether the exosomal mRNA content had changed. This was evaluated by isolation of RNA followed by Affymetrix microarray analysis. This analysis was performed on RNA from both the exosomes and their donor cells. The Affymetrix microarray analysis confirmed our previously published results [8], that there is no correlation between cellular mRNA and the exosomal mRNA indicating a difference in mRNA content (Figure 4d). In addition, this lack of correlation was also seen between donor cell and exosomal mRNA under oxidative stress (Figure 4e). Importantly, a difference in mRNA content was observed between exosomes harvested from the different conditions (Figure 4f). Furthermore, the results also showed a slight difference in gene expression in cells cultured under normal conditions compared to oxidative stress (Figure 4c).

Interestingly, the relationship between significant regulatory transcripts found in exosomes from normal conditions and from oxidative stress were shown to change substantially in exosomes, although in cells this relationship between the two conditions were similar (Figure 5).

The top 20 up- and down-regulated genes in exosomes harvested from cells cultured under oxidative stress are shown in Table 1 and 2 respectively.

**UV- light eliminates the protective effect of exosomes against oxidative stress**

Since the mRNA content of exosomes differs substantially in exosomes released under oxidative stress compared to exosomes released under normal conditions, and as exosomes released under oxidative stress can induce a resistance against oxidative stress in recipient cells, we hypothesized that the conditioning effect could be mediated by the RNA content in exosomes. To test this hypothesis, exosomes harvested from oxidative stress were exposed to UV-light (254 nm) for 1 h, as UV-light inactivates RNA functions [42–43]. As controls, exosomes from both normal and stressed conditions were treated in parallel, but without exposure to UV-light. After the UV-light exposure, the exosomes were added to untreated cultures of recipient cells which were then exposed to oxidative stress, as in the previous experiments, and any influence on cell viability was determined at 0, 2 and 12 h.
The results revealed that exosomes exposed to UV-light lost their protective effect on the viability of recipient cells exposed to oxidative stress at the 12 h time point (Figure 6).

Discussion

This study shows that exosomes, released from mast cells exposed to oxidative stress, have the capacity to communicate a protective signal to recipient cells exposed to subsequent oxidative stress, resulting in reduced cell death. The mRNA content of exosomes produced under oxidative stress differs extensively from both the mRNA in the donor cell and in the exosomes produced by cells cultured under normal conditions. UV-light exposure, which damages nucleic acids [42–44] and proteins [45], eliminate the exosomal protective signal, which may suggest that the exosomal shuttle of RNA at least partly mediate the observed effect.

Exosomes harvested from different cells under different situations have been shown to mediate a multitude of biological effects, including antigen presentation [6,21], induction of apoptosis [46], and promotion of cancer cell growth [47] as a few examples. The current study adds to the list of biological functions of exosomes, proving that exosomes produced during oxidative stress mediate protective signals to the same stress in other cells. Thus, we observed that exosomes, released by cells

![Figure 2. MC/9 cells pre-treated with exosomes released under oxidative stress obtain a resistance to oxidative stress.](image1)

**Figure 2.** MC/9 cells pre-treated with exosomes released under oxidative stress obtain a resistance to oxidative stress. Time course of viability of MC/9 cells (n = 6) (%) after exposure to oxidative stress (H2O2 125 μM) when pre-treated with exosomes derived from other MC/9 cells that were either exposed (oxi exo) or not exposed (norm exo) to H2O2 at the same concentration. Treatment of MC/9 cells with exosomes released under oxidative stress increased viability with approximately 15-20% at different time points after the initiation of H2O2 exposure. ***p<0.001.

![Figure 3. Cells exposed to H2O2 show an increase in oxidized proteins, whereas exosomes do not.](image2)

**Figure 3.** Cells exposed to H2O2 show an increase in oxidized proteins, whereas exosomes do not. Oxidized proteins (% intensity) in MC/9 cells (a) and their released exosomes (b) after exposure to vehicle or H2O2 (125 μM) for 24 h (n = 5). Oxidative stress significantly increased the relative amount of oxidized proteins in cells, but did not significantly affect the relative amount of oxidized proteins in exosomes. *p<0.05.

![Additional figure](image3)
Figure 4. Scatter plots of relationships between mRNA signals in MC/9 cells and exosomes. a) Reproducibility comparison of mRNA signals between two different cell cultures of MC/9 cells (sample norm cell A and norm cell B) under normal conditions (norm). b) Reproducibility of comparison of mRNA signals between exosomes derived from two different cell cultures of MC/9 cells (sample norm exo A and norm exo B) under normal conditions (norm). c) Relationship between mean mRNA signals between MC/9 cells (samples norm cell A–D and oxi cell A–D) that have been exposed to vehicle (norm) or 125 μM H₂O₂ (oxi) for 24 h. d) Relationship between mean mRNA signals in MC/9 cells and their released exosomes (samples norm cell A–D and norm exo A–D) under normal conditions (norm). e) Relationship between mean mRNA signals in MC/9 cells and their released exosomes (samples oxi cell A–D and oxi exo A–D) after H₂O₂ exposure for 24 h (125 μM) (oxi). f) Relationship between mean mRNA signals in exosomes released from MC/9 cells (samples norm exo A–D and oxi exo A–D) after exposure to vehicle (norm) or H₂O₂ (125 μM) (oxi) for 24 h.
doi:10.1371/journal.pone.0015353.g004

Figure 5. Relationship between significantly changed transcripts in MC/9 cells and exosomes, from normal and oxidative stress conditions. Significantly regulated transcripts found in cells and exosomes (n = 4, all present) from both normal conditions and from H₂O₂ (125 μM, 24 h) induced oxidative stress. The majority of the cellular transcripts are the same in both cells grown under normal conditions and in cells grown under oxidative stress, as shown by the grey field, and only a small percentage of the transcripts change depending on the condition. The blue field shows the transcripts that are only expressed in cells grown under normal conditions and the red field shows transcripts only expressed in cells grown under oxidative stress conditions. However, the significantly regulated transcripts in exosomes change vastly depending on the condition compared to the cells.
doi:10.1371/journal.pone.0015353.g005
The effects of exosomes, we were careful to choose a dose of H$_2$O$_2$ to induce oxidative stress resulted in an increased relative amount of introduced carbonyl groups in the proteins of exposed cells. To reduce the functionality of the RNA in the exosomes, we exposed the exosome fraction to UV-C radiation, as this treatment cannot be excluded. A biological role of exosomal proteins in this experiment is known to have a damaging effect on nucleic acids [42–44,50]. Since the exosomal RNA content changed extensively under conditions of oxidative stress and because we have previously shown that the exosomal RNA content in the exosomes is closely regulated depending on a cell’s biological state or function. This result confirms our previous conclusion that the exosomal mRNA content in the exosomes is not a random sample of the cellular mRNA [8], as it differed substantially from the donor cell mRNA regardless of the cell culture conditions.

In the current study, we hypothesized that the exosomal RNA content changes, and that this change is not only dependent on the cell origin but also on the condition under which they have been produced and released under, in this case normal conditions and oxidative stress. Indeed, the Affymetrix microarray analysis show substantial differences in mRNA gene expression in exosomes compared to their donor cells, both from cells with and without exposure to oxidative stress. Also, the exosomal mRNA content substantially differed in exosomes harvested from cells grown under the different conditions, arguing that the RNA content in exosomes is closely regulated depending on a cell’s biological state or function.

Table 1. Induced genes in exosomes released under oxidative stress.

| Gene symbol/Gene name | Mean signal norm exo | Mean signal oxi exo | Fold change | p-value |
|-----------------------|----------------------|---------------------|-------------|---------|
| [Vsig1] V-set and immunoglobulin domain containing 1 | 200 | 663 | 3.3 | 1.66E-03 |
| [Top1] topoisomerase (DNA) I | 540 | 1402 | 2.6 | 4.63E-02 |
| [Cebp2] chemokine binding protein 2 | 356 | 897 | 2.5 | 3.32E-02 |
| [0610010K06Rik] RIKEN cDNA 0610010K06 gene | 383 | 966 | 2.5 | 1.32E-02 |
| [Krt11] KRT11, ankyrin repeat containing | 325 | 811 | 2.5 | 2.14E-02 |
| [D230019N24Rik] RIKEN cDNA D230019N24 gene | 426 | 1015 | 2.4 | 5.42E-03 |
| [Amy2a1] amylase 2a1, pancreatic | 349 | 827 | 2.4 | 7.77E-04 |
| [Lba1] lupus brain antigen 1 | 532 | 1230 | 2.3 | 3.92E-02 |
| [Zfp385c] zinc finger protein 385C | 585 | 1349 | 2.3 | 1.73E-02 |
| [2700057C20Rik] RIKEN cDNA 2700057C20 gene | 528 | 1213 | 2.3 | 2.52E-03 |
| [Ptar1] protein prenyltransferase alpha subunit repeat containing 1 | 688 | 1557 | 2.3 | 2.55E-02 |
| [Smad3] MAD homolog 3 (Drosophila) | 593 | 1339 | 2.3 | 2.35E-02 |
| [2810002D19Rik] RIKEN cDNA 2810002D19 gene | 239 | 530 | 2.2 | 1.10E-02 |
| [Phf6] PHD finger protein 6 | 527 | 1154 | 2.2 | 1.06E-02 |
| [Hsd17b11] hydroxysteroid (17-beta) dehydrogenase 11 | 386 | 813 | 2.1 | 3.18E-02 |
| [6720457D02Rik] RIKEN cDNA 6720457D02 gene | 963 | 2023 | 2.1 | 4.95E-02 |
| [Yipf7] Yip1 domain family, member 7 | 705 | 1463 | 2.1 | 1.59E-02 |
| [Mep1a] meprin 1 alpha | 375 | 765 | 2.0 | 3.87E-02 |
| [Sox15] SRY-box containing gene 15 | 318 | 648 | 2.0 | 4.73E-02 |
| [4930473M17Rik] RIKEN cDNA 4930473M17 gene | 421 | 845 | 2.0 | 3.03E-03 |

This table shows the 20 most induced mRNA transcripts in exosomes derived from MC/9 cells exposed to oxidative stress (H$_2$O$_2$, 1, 2, 5 μM for 24 h, oxi exo) compared to exosomal mRNA transcripts after exposure of cells to vehicle (norm exo). A fold-change of e.g. 2 indicated that the gene is 2 fold up-regulated in the exosomes derived from cells exposed to oxidative stress.

doi:10.1371/journal.pone.0015353.t001

As shown in previous studies, we confirm that exposure of mast cells to H$_2$O$_2$ results in reduced cell viability in vitro [33]. To study the effects of exosomes, we were careful to choose a dose of H$_2$O$_2$ that resulted in a moderate degree of cell death, to be able to study any up or down regulating effects of exosomes. It is well known that oxidative stress can lead to various cell damage such as lipid peroxidation, nucleic acids oxidation and protein oxidation [41,48–49]. The results of protein oxidation by ROS are many, including cleavage of peptide bonds, cross-linkage reactions and generation of carbonyl derivates [49]. Interestingly, the dose of H$_2$O$_2$ to induce oxidative stress resulted in an increased relative amount of introduced carbonyl groups in the proteins of exposed cells, but not in the proteins of exosomes that they released. Thus, the cells seem to be extensively affected themselves by the oxidation process, unlike the exosomes. We suggest that the cells may actively protect the exosomes from containing damaged proteins by specifically packaging the exosomes with undamaged proteins. This data also argues that the conditioning signal mediated by exosomes released during oxidative stress is not mediated by oxidized exosomal proteins per se.

In previous work, we have shown that the RNA content in exosomes differs extensively from the donor cell’s RNA [8]. The current study, we hypothesized that the exosomal RNA content changes, and that this change is not only dependent on the cell origin but also on the condition under which they have been grown under oxidative stress, reduce cell death. Consequently, we show that the oxidative stress alters the biological function of exosomes released from mast cells, which further argues that these vesicles can communicate important regulatory signals from one cell to another.
Materials and Methods

MC/9 cell culture, oxidative stress treatment and exosome isolation

MC/9 cells (ATCC, Manassas, VA) were cultured in Dulbecco’s Modified Eagle’s Medium, 10% fetal bovine serum (FBS), 100 µg/ml penicillin-streptomycin, 2 mM L-glutamine, 0.05 mM 2-mercaptoethanol (all from Sigma-Aldrich, St Louis, MO, USA) and 10% Rat T-Stim (BD Biosciences, Erembodegem, Belgium), at 37°C and 5% CO2. The FBS and Rat T-Stim contain exosomes. To remove these exosomes, FBS and Rat T-Stim were ultracentrifuged at 120,000 x g for 90 min, 4°C (Ti45 rotor, Beckman Coulter, Brea, CA, USA). To induce oxidative stress, cells were exposed to 125 µM H2O2 (Sigma-Aldrich) for 24 h under culture conditions. For isolation of exosomes, MC/9 cell suspension was centrifuged for 10 min at 300 g, to pellet the cells, and the exosomes were prepared from the supernatant. The exosomes were purified by ultracentrifugation in a Beckman Ultracentrifuge (rotor Ti45). The exosomes were then pelleted by ultracentrifugation at 120,000 x g, 70 min, 4°C.

Table 2. Repressed genes in exosomes released under oxidative stress.

| Gene symbol/Gene name | Mean signal norm exo | Mean signal oxi exo | Fold change | p-value |
|-----------------------|----------------------|---------------------|-------------|---------|
| [Ctnna1] catenin (cadherin associated protein), alpha 1 | 1417 | 176 | -8.0 | 1.83E-02 |
| [Pig9] phosphatidylinositol glycan anchor biosynthesis, class Q | 1661 | 212 | -7.8 | 4.15E-02 |
| [Cct2] chaperonin containing Tcp1, subunit 2 (beta) | 2094 | 278 | -7.5 | 9.33E-04 |
| [Rfcr] replication factor C (activator 1) 4 | 1115 | 149 | -7.5 | 5.48E-03 |
| [Gnas] GNAS (guanine nucleotide binding protein, alpha stimulating) complex locus | 2193 | 324 | -6.8 | 5.96E-03 |
| [Ttc3] tetratricopeptide repeat domain 3 | 1701 | 253 | -6.7 | 4.35E-02 |
| [Laptm5] lysosomal-associated protein transmembrane 5 | 15814 | 2461 | -6.4 | 9.80E-03 |
| [Gabaprl1] gamma-aminobutyric acid (GABA) A receptor-associated protein-like 1 | 1180 | 188 | -6.3 | 1.61E-03 |
| [Ipob] importin 4 | 1706 | 276 | -6.2 | 2.10E-02 |
| [Dnpep] aspartyl aminopeptidase | 5271 | 871 | -6.1 | 8.76E-03 |
| [Lmna] lamin A | 1918 | 329 | -5.8 | 1.52E-02 |
| [Sor3] signal sequence receptor, gamma | 5227 | 912 | -5.7 | 1.96E-02 |
| [Qars] glutaminyl-tRNA synthetase | 1905 | 341 | -5.6 | 1.54E-04 |
| [Gsn] gelsolin | 4203 | 811 | -5.2 | 1.53E-02 |
| [Arap3] ArfGAP with RhogAP domain, ankyrin repeat and PH domain 3 | 2416 | 470 | -5.1 | 6.95E-03 |
| [Med22] mediator complex subunit 22 | 3454 | 679 | -5.1 | 3.06E-02 |
| [Csnk1d] casein kinase 1, delta | 1383 | 275 | -5.0 | 6.96E-03 |
| [Coror7] coronin 7 | 2339 | 465 | -5.0 | 1.18E-02 |
| [Lasp1] LIM and SH3 protein 1 | 3478 | 698 | -5.0 | 5.03E-05 |
| [Ric8] resistance to inhibitors of cholinesterase 8 homolog (C. elegans) | 1956 | 382 | -4.8 | 4.88E-02 |

This table shows the 20 most repressed mRNA transcripts in exosomes derived from MC/9 cells exposed to oxidative stress (H2O2, 125 µM for 24 h, oxi exo) compared to exosomal mRNA transcripts after exposure of cells to vehicle (norm exo). A fold-change of e.g. 2 indicated that the gene is 2 fold down-regulated in the exosomes derived from cells exposed to oxidative stress.

doi:10.1371/journal.pone.0015353.t002

It is clear that exosomes harvested from different cells and under different conditions have vastly diverse effects in different cell systems. This suggests that exosomes can have a multitude of effects in vivo, depending on how and where they were produced. Many studies suggest that the core protein content of exosomes in fact are conserved [3,20], whereas the RNA content in exosomes, according to our current findings, can change extensively under different conditions. It is therefore possible that many of the diverse functions of exosomes reported in different studies are in fact mediated by different RNA signals that are shuttled between cells by exosomes. The current study therefore further emphasizes the putative biological regulatory importance of the shuttling of RNA between cells by exosomes.

In conclusion, in this study we have shown that exosomes that are produced by cells exposed to oxidative stress have the ability to induce tolerance to oxidative stress in another cell. This effect is associated with changed exosomal mRNA content that can be attenuated by reduced RNA activity through exposure to UV-light. This shows, for the first time, that the exosomal shuttle of RNA can fundamentally change the biological function of a recipient cell. When functions of exosomes are pursued, the role of their RNA content should be carefully considered.

Materials and Methods

MC/9 cell culture, oxidative stress treatment and exosome isolation
chip analysis (Affymetrix). Gene expression profiles were analyzed using the MAS5.0 software (Affymetrix).

Accession Number

The microarray data have been deposited in NCBI's Gene Expression Omnibus (GEO). Details can be found at http://www.ncbi.nlm.nih.gov/geo (the GEO accession number is: GSE24886).

Transfer experiment and cell viability analysis

All exosomes were isolated (n = 6) from MC/9 donor cells exposed to H2O2 (125 μM) or vehicle (complete medium) for 24 h and redissolved in complete medium. All of the exosomes collected from the supernatant from the donor cell cultures were added to the MC/9 recipient cells in the ratio of 1.7:1. This approach was taken to ensure that all exosomes and their content were transferred, which would better reflect the true biological state as opposed to a small subset. The recipient cells and exosomes were then incubated for 3 h under normal culture conditions. The recipient cells were subsequently challenged with H2O2 (125 μM) and harvested after 0, 2, 12 and 24 h. The cell viability was assessed by using the trypan blue dye exclusion method.

Detection of oxidized proteins

The total protein was extracted from cells and exosomes (n = 5) using modified RIPA buffer [51] and sonication. Cell debris was removed by centrifugation. Detection and quantification of oxidized proteins was performed using the OxyBlot™ oxidized protein detection kit (Millipore, Billerica, MA, USA) according to the manufacturer’s recommendations. In brief, the protein carbonyl groups, which are a consequence of the oxidative stress modification, were derivatized. Equal amounts of protein (15–20 μg) were then separated on polyacrylamide gels, transferred onto nitrocellulose membranes (Bio-Rad, Hercules, CA, USA) and blotted using antibodies specific to the OxyBlot™ kit. Enhanced chemiluminescence (GE Healthcare, Uppsala, Sweden) and Quantity One® software (Bio-Rad) was then used for visualisation and relative quantification.

Exposure of exosomes to UV-light and subsequent transfer

All exosomes were isolated (n = 6) from MC/9 donor cells exposed to H2O2 (125 μM) or vehicle (complete medium) for 24 h and resuspended in PBS. Exosomes isolated from cells exposed to H2O2 were then subjected to UV-light (254 nm) for 1 h at 0–4°C. As controls, exosomes released by cells exposed to H2O2 or vehicle, not subjected to UV-light, were kept at 4°C for 1 h. The exosomes were then added to MC/9 recipient cells in the ratio of 1.7:1 between donor cells and recipient cells and incubated for 3 h under normal culture conditions. The recipient cells were subsequently challenged with H2O2 (125 μM) and harvested after 0, 2 and 12 h. The cell viability was assessed by using the trypan blue dye exclusion method.

Statistical analysis

Where appropriate, data are expressed as mean ±SEM. Statistical analysis was performed by one-way ANOVA test when comparing more than two groups and paired t-test (two tailed) analyses were used when comparing two conditions (SPSS for Windows® version 17.0). Differences in gene expression between normal conditions and oxidative stress were assessed with paired t-test (two tailed). A probability less than 0.05 was accepted as statistically significant.
Acknowledgments

We thank Sweene Microarray Resource Centre at Lund University for assistance with the Affymetrix microarray processing.

References

1. Pan BT, Teng K, Wu C, Adam M, Johnstone RM (1985) Electron microscopic evidence for externalization of the transferrin receptor in vesicular form in sheep reticulocytes. J Cell Biol 101: 942–948.

2. Thery C, Zitvogel L, Amigorena S (2002) Exosomes: composition, biogenesis and function. Nat Rev Immunol 2: 569–579.

3. Keller S, Sanderson MP, Stoeck A, Altevogt P (2006) Exosomes: From biogenesis and secretion to biological function. Immunol Lett 107: 102–106.

4. Pan BT, Johnstone RM (1983) Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro. Selective externalization of the receptor. Cell 33: 967–978.

5. Johnstone RM, Adam M, Hammond JR, Orr L, Turbide C (1987) Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). J Biol Chem 262: 9412–9420.

6. Raposo G, Nijman HW, Stoorvogel W, Ljejendeleker R, Harding CV, et al. (1996) Lymphocytes secrete antigen-presenting vesicles. J Exp Med 183: 1101–1112.

7. Raposo G, Tenza D, Mecheri S, Peronet R, Bonnetor C, et al. (1997) Accumulation of Major Histocompatibility Complex Class II Molecules in Mast Cell Secretory Granules and Their Release upon Degranulation. Mol Biol Cell 8: 2631–2645.

8. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, et al. (2007) Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 9: 535–545.

9. Zitvogel L, Regnault A, Lozier A, Wollers, J, Flamant C, et al. (1998) Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell derived exosomes. Nat Med 4: 394–400.

10. Thery C, Regnault A, Garin J, Wollers, J, Zitvogel L, et al. (1999) Molecular Characterization of Dendritic Cell-derived Exosomes. Selective Accumulation of the Heat Shock Protein hsc73. J Cell Biol 147: 599–610.

11. Blanchard N, Lankar D, Faure F, Regnault A, Dumont C, et al. (2002) TCR Activation of Human T Cells Induces the Production of Exosomes Bearing the TCR/CD3/ζ complex. J Immunol 168: 3241–3248.

12. Wollers J, Lozier A, Raposo G, Regnault A, Thery C, et al. (2002) Tumor-derived exosomes are a source of shared tumor rejection antigens for CTL cross-presentation. Nat Med 8: 295–303.

13. Andre F, Schattz NEC, Movassagh M, Flamant C, Pautier P, et al. (2002) Malignant effusions and immunogenic tumor-derived exosomes. The Lancet 359: 295–305.

14. Van Niel G, Raposo G, Candall C, Bossac M, Herbstor R, et al. (2001) Intestinal epithelial cells secrete exosome-like vesicles. Gastroenterology 121: 337–349.

15. Caby M-P, Lankar D, Vincendeau-Scherrer C, Raposo G, Bonnetor C (2005) Exosomal-like vesicles are present in human blood plasma. Int Immunol 17: 879–887.

16. Bai S, Shen R-F, Knepper MA (2004) Identification and proteomic profiling of exosomes in human urine. Proc Natl Acad Sci U S A 101: 1161–1168.

17. Palanisamy V, Sharma S, Deshpande A, Zhou H, Gimzewski J, et al. (2010) Exosomal Communication during Oxidative Stress. PLoS One 5: e8577.

18. Adnyre C, Johansson SM, Qazi KR, Filen J, Labesmaa R, et al. (2007) Exosomes with Immune Modulatory Features Are Present in Human Breast Milk. J Immunol 179: 1609–1620.

19. Adnyre C, Johansson SM, Qazi KR, Filen J, Labesmaa R, et al. (2007) "Tolerosomes" are produced by intestinal epithelial cells. J Exp Med 183: 2631–2645.

20. Denzer K, van Eijk M, Kleijmeer MJ, Jakobson E, de Groot C, et al. (2000) Follicular Dendritic Cells Carry MHC Class II-Expressing Microvesicles at Their Surface. J Immunol 165: 1329–1337.

21. Adnyre C, Johansson SM, Qazi KR, Filen J, Labesmaa R, et al. (2007) "Tolerosomes" are produced by intestinal epithelial cells. J Exp Med 183: 2631–2645.

22. Adnyre C, Johansson SM, Qazi KR, Filen J, Labesmaa R, et al. (2007) "Tolerosomes" are produced by intestinal epithelial cells. J Exp Med 183: 2631–2645.

23. Denzer K, van Eijk M, Kleijmeer MJ, Jakobson E, de Groot C, et al. (2000) Follicular Dendritic Cells Carry MHC Class II-Expressing Microvesicles at Their Surface. J Immunol 165: 1329–1337.

24. Morelli AE, Larregina AT, Shafesky WJ, Sullivan MLG, Stolz DB, et al. (2004) Endocytosis, intracellular sorting, and processing of exosomes by dendritic cells. Blood 104: 1257–1266.

25. Tian T, Wang Y, Yang H, Zhu Z, Xiao Z (2010) Visualizing of the cellular uptake and intracellular trafficking of exosomes by live-cell microscopy. J Cell Biochem.