

# Nanovesicles Mediated Tumor Tolerance in Dalton's Ascites Lymphoma Mice

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## Abstract

Shedding of microvesicles from live cells with 40- 100 nm size is termed as exosomes or nanovesicles. They are secreted by various types of cells such as mast cells, dendritic cells, epithelial cells and so on. Inward interaction of plasma membrane along with its intracellular components results in the formation of budded microvesicles. These nanovesicles can also act as signalosomes, since they carry genetic information such as exosomal shuttle RNA, miRNA, DNA and proteins. Exosomes mediated signals are as similar as their cellular origin and it also transfers the information efficiently through cellular communication. Taking advantage of this peculiar function, cancer cells derived exosomes triggers cellular proliferation and attains malignancy by transferring the signals even to distant cells. Cell cycle dysregulation was well documented in Dalton's ascites lymphoma mice and intra peritoneal injection of  $1 \times 10^6$  cells from DAL mice could induce metastasis in normal Swiss albino mouse within 10 days. To conclude, exchange of cellular signals by cancer cells derived exosomes efficiently induces metastasis in DAL mice. Consequently, this review will emphasize the role of exosomes in regulating cancer stemness and its microenvironment.

**Keywords:** Nanovesicles; Stemness; Dalton's ascites lymphoma (DAL); Tumor microenvironment

## Composition and Functions of Exosomes

Membrane bound vesicles which are 40-100 nm size are termed as exosomes. It was released as a result of multivesicular body fusion with plasma membrane [1]. Rose Johnstone in 1987 noticed the release of vesicles from cultured monolayer cells which contains significant enzyme activity and also escapes from the lysosomal degradation process [2]. Structure of exosomes contains tetraspanins (CD63, CD81, CD9), luminal protein TSG 101, integrins, and Rab proteins [3]. Apart from proteins, they are also enriched with lipids and its derivatives e.g. sphingolipids, phosphoglycerides, cholesterol, ceramide and phosphoglycerides [4]. Exosomal protein transport can be facilitated by the presence of chaperons otherwise known as heat shock protein (Hsp70) [5]. Functions of exosomes mainly depend on their parental cells or cellular origin from where it has been derived. Physiological circumstances also influence exosomal release and its function. Initially, it was presumed that exosomes could act only as garbage machinery, but later it has been proved that exosomes could act as a cell to cell messenger in a microenvironment and also at distant sites (Figure 1) [6].

Most of the body fluids such as saliva, serum, breast milk, lymph, urine, amniotic fluid, semen are known to possess exosomes and through systemic circulation blood plasma derived exosomes might be transported to various organs [7-9]. Till date the cellular interaction with exosomes is not discussed clearly and it may have receptor-ligand interactions as similar as in the cell- cell communication process [10]. Tumor derived MVs may induce epigenetic changes in the target cells by transferring genetic information. It not only transfers surrounding determinants but also transfer signals from tumor cells to normal cells. This effect is executed by horizontal transfer of exosomal shuttle mRNA and the biologically active proteins are expressed in the recipient cells [11]. Further, exosomes from a mouse or human mast cells could be transferred to other mouse and human mast cells. Further the transfer of genetic material has been confirmed by the

presence of new proteins in recipient cells [12]. Exosome mediated transfer of genetic information has a major role in maintaining the cellular microenvironment and its stemness.

## Exosomes and its Secretion

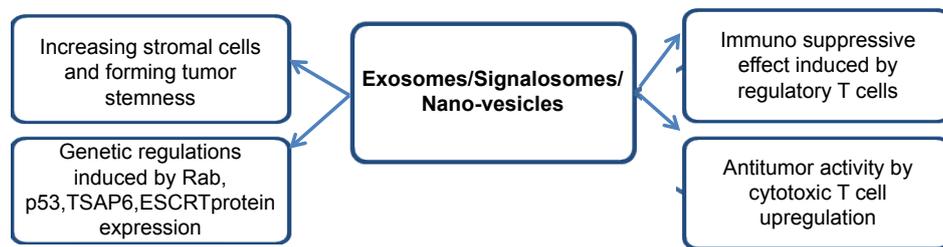
Firstly, exosomes have been believed as vesicles to dispose the unwanted proteins from cells, but later it has been confirmed that it also communicates with its microenvironment. Basically two different pathways are involved in exosome secretion i.e. by Trans-Golgi network and the other one is through inducible release from multivesicular bodies. From the literature survey, it has been confirmed that stress or DNA damage leads to the activation and or upregulation of p53 a tumor suppressor protein, which thereby initiates the tumor suppressor activated protein pathway (TSAP-6) followed by ESCRT (Endosomal Sorting Complex Required for Transport) proteins expression and finally it increases the exosome secretion [13]. Though the active form of p53 is not expressed, over expression of TSAP-6 by Rab/GTPase proteins could increase the exosomes production [14]. Hence p53 regulates exosome production through TSAP-6 pathway. Another possibility of increasing the exosomes production could be done by up regulating Rab/GTPase proteins to regulate the expression of TSAP-6 proteins. In most of the cancer cells, mutated Rab-GTPase

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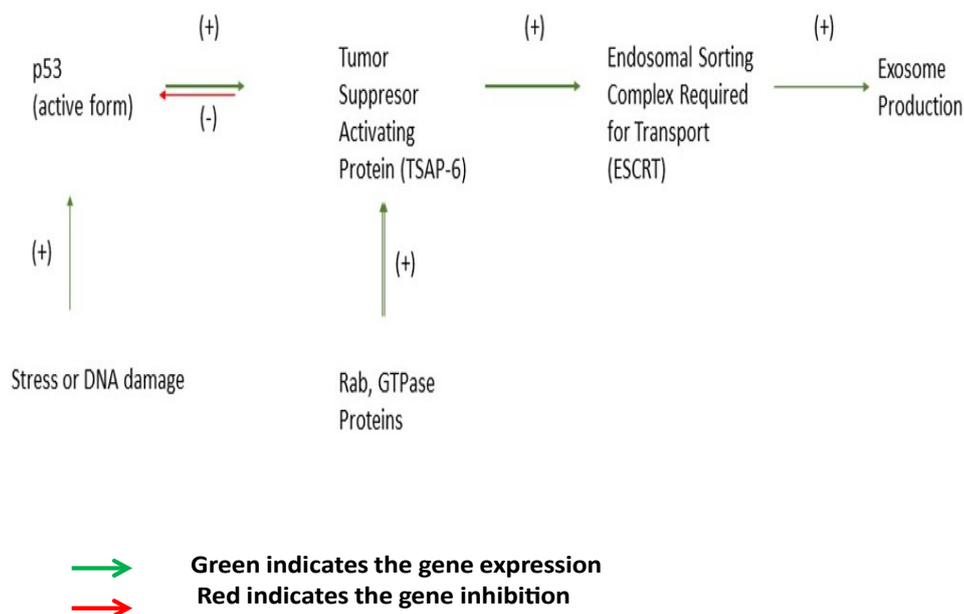
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**Figure 1:** Overview of exosomal regulation in inducing cancer through various mechanisms.



**Figure 2:** Schematic representation of Exosomal synthesis and its regulation.

and its over expression has been reported [15]. Hence the exosomes secretion in tumor cells and its crosstalk between vicinity cells could facilitate the tumor microenvironment (Figure 2).

### Cell- cell Interactions

Very recently, exosomes are considered to play an essential cellular function by the presence of its shuttle RNA and miRNA [16]. Bilipid layer of exosomes also contains integrins which assist them in a strong adhesion with target cells. The transformation of genetic material occurred horizontally from exosomes to the target cells and thereby it influences the target cell functions [17,18]. Based on their cellular origin and the parental cell physiology, these signalosomes possess a wide range of functions. Exosomal shuttle mRNA is capable of travelling distant sites to reach the recipient cells and thereby it influence the recipient cell's protein production [19]. Extracellular release of exosomes enables them to interact with the target cells and leads to physiological changes [20]. The bottom line of exosomal interactions with recipient cells may occur through three ways, i.e. fusion of target cell plasma membrane with exosomes leads to their release into the cells [21]. The second one is based on the ligand receptor binding process [22] and the third one is through endocytosis or pinocytosis process [23]. Mostly, these nanovesicles internalize into

recipient cells by the fusion of its plasma membrane when compared to endocytosis process [24].

### Exosomes in Pathological Conditions

At the outset, in 2001 Taylor confirmed the release of microvesicles from ovarian cancer cells [25]. A wide range of pathological conditions i.e. metastasis of cancer, cardiovascular and degenerative diseases is well accompanied with exosomes concentration and function. Cancer cells derived exosomes are efficient to transfer signals as tumor antigens to dendritic cells and thereby induce immune responses [20]. But it can also induce immunosuppressive effect by inducing regulatory T cells differentiation or by activating apoptosis in cytotoxic T- cells [26]. It was well documented that tumor cells derived exosomes promotes angiogenesis and metastasis process [19]. Exosomes exert anti-tumoral immunity by the presence of their tumor specific antigens in the other way it releases soluble immune suppressive factors in the form vesicles into their microenvironment [27]. Hence these nanovesicles induce significant tumor microenvironment in cancer cells. In addition, tumor derived exosomes also upregulates apoptotic inhibitory proteins and enhances tumor progression. Glioblastoma derived exosomes showed the transfer of genetic material and proteins from tumor cells to neighboring cells that facilitates the tumor invasion and

metastasis process [28,29]. Experimental evidence on gastro-intestinal cancer induced rat model suggested the enrichment of cell adhesion molecules such as CD63, CD81 and CD9 in tumor derived exosomes. Presence of such structural tetraspanins in tumor exosomes assists them to promote angiogenesis and metastasis [30]. Solid tumor cells derived exosomes increased the stromal cell populations significantly and this peculiar property facilitates the tumor invasion, angiogenesis and metastasis [31]. Recent report on cancer cells derived exosomes depicted that exosomal DNA could induce cellular proliferation since it resembles the parental genetic mutations. In addition, they also emphasized that packaging of exosomal content varies with different type of cancer cells such as leukemia, melanoma, breast, prostate and lung etc. [32].

### Case Study: Dalton's Ascites Lymphoma (DAL)

The animals (Swiss albino strain, male  $\pm$  30 g body weight per mouse) were fed with pellet feed (Sai Durga Feeds Ltd, Bangalore) and water ad libitum. They were maintained under normal lighting regimen (12 L:D) with controlled temperature ( $25 \pm 5^\circ\text{C}$ ) throughout the experimental period. Swiss albino mice provide a convenient model, since intra peritoneal injection of ascites cells of DAL mice ( $1 \times 10^6$  cells/ mouse around 30 g body weight) into abdominal cavity of normal healthy recipient mouse leads to tumor allograft and proliferation was observed after 72 hrs. Morphological differences with increased tumor volume and body weight was observed in DAL induced mice (Figure 3). The study was performed with the guidelines of Institutional Animal Ethics Committee (IAEC), Bharathidasan University, India [33].

Aggressive tumor development in DAL was associated with inhibition of humoral and cell mediated immunity [34]. It has been studied in DAL tumor cells that B-cells dominate cell mediated immune responses thereby activating anti-apoptotic pathway for high cellular proliferation [35]. Initiation and progression of DAL tumor cells occurred through reactive oxygen metabolites under hypoxic condition. In general, exponential decrease in cellular immunity directs to high risk of lymphomas and melanomas [36]. Indeed, it was confirmed that DAL tumor cells derived exosomes remains as a close replica of its parental cells and possess similar functions which confers a significant immunosuppressive effect, when it was injected into a normal mouse. These signalosomes could also transfer their genetic information through shuttle RNA, miRNA and proteins to the adjacent and distant cells [29]. Beyond these cell to cell interactions in DAL mice, exosomes cause phenotypic changes by enhancing its tumor volume and the stromal cell populations [33]. To conclude, preliminary evidences of this case study confirmed that the ascites fluid from DAL mice contain notable tumor derived signalosomes or nanovesicles, which shapes the microenvironment and promotes tumor stemness

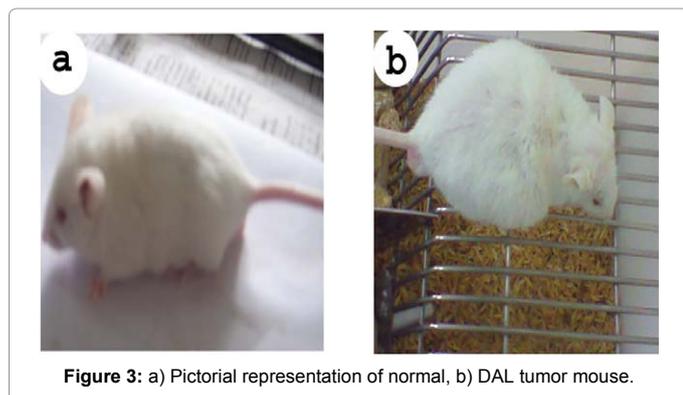


Figure 3: a) Pictorial representation of normal, b) DAL tumor mouse.

and metastasis process. Consequently a better understanding on tumor derived exosomes will direct a new biomarker for cancer diagnosis and therapeutics.

### Conflict of Interest

Authors declare that there is no conflict of interest concerning the publication of this review article.

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### References

1. Denzer K, Kleijmeer MJ, Heijnen HF, Stoorvogel W, Geuze HJ (2000) Exosome: from internal vesicle of the multivesicular body to intercellular signaling device. *J Cell Sci* 113: 3365-3374. [PubMed]
2. 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. [PubMed]
3. Carayon K, Chaoui K, Ronzier E, Lazar I, Bertrand-Michel J, et al. (2011) Proteolipidic Composition of Exosomes Changes during Reticulocyte Maturation. *J Biol Chem* 286: 34426-34439. [PubMed]
4. Subra C, Laulagnier K, Perret B, Record M (2007) Exosome lipidomics unravels lipid sorting at the level of multivesicular bodies. *Biochimie* 89: 205-212. [PubMed]
5. Conde-Vancells J, Rodriguez-Suarez E, Gonzalez E, Berisa A, Gil D (2010) Falcon-Pere, Candidate biomarkers in exosome-like vesicles purified from rat and mouse urine samples. *Proteomics Clin Appl* 4: 416-425. [PubMed]
6. F evrier B, Raposo G (2004) Exosomes: endosomal-derived vesicles shipping extracellular messages. *Curr Opin Chem Biol* 16: 415-421. [PubMed]
7. Keller S, Rupp C, Stoeck A, Runz S, Fogel M, et al. (2007) CD24 is a marker of exosomes secreted into urine and amniotic fluid. *Kidney Int* 72: 1095-1102. [PubMed]
8. L asser C, Alikhani VS, Ekstr om K, Eldh M, Paredes PT, et al. (2011) Human saliva, plasma and breast milk exosomes contain RNA: uptake by macrophages. *J Transl Med* 9: 1-8. [PubMed]
9. Caby MP, Lankar D, Vincendeau-Scherrer C, Raposo G, Bonnerot C (2005) Exosomal-like vesicles are present in human blood plasma. *Int Immunol* 17: 879-887. [PubMed]
10. Admyre C, Johansson SM, Paulie S, Gabrielsson S (2006) Direct exosome stimulation of peripheral human T cells detected by ELISPOT. *Eur J Immunol* 36: 1772-1781. [PubMed]
11. Hergenreider E, Heydt S, Tr equer K, Boettger T, Horrevoets AJ, et al. (2012) Boon & Stefanie Dimmeler, Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs. *Nat Cell Biol* 14: 249-256. [PubMed]
12. Valadi H, Ekstr om K, Bossios A, Sj strand 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: 654-659. [PubMed]
13. Tamai K, Tanaka N, Nakano T, Kakazu E, Kondo Y, et al. (2010) Exosome secretion of dendritic cells is regulated by Hrs, an ESCRT-0 protein. *Biochem Biophys Res Comm* 399: 384-390. [PubMed]
14. Yu X, Harris SL, Levine AJ (2006) The Regulation of Exosome Secretion: a Novel Function of the p53 Protein. *Cancer Res* 66: 4795-4801. [PubMed]
15. Ostrowski M, Carmo NB, Krumeich S, Faret J, Raposo G, et al. (2010) Rab27a and Rab27b control different steps of the exosome secretion pathway. *Nat Cell Biol* 12: 19-30. [PubMed]
16. Kosaka N, Iguchi H, Ochiya T (2010) Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. *Cancer Sci* 101: 2087-2092. [PubMed]
17. Clayton A, Turkes A, Dewitt S, Steadman R, Mason MD, et al. (2004) Adhesion

- and signalling by B cell derived exosomes: The role of integrins. *FASEB J* 9: 977-979. [[PubMed](#)]
18. Mittelbrunn M, Gutiérrez-Vázquez C, Villarroya-Beltri C, González S, Sánchez-Cabo F (2011) Unidirectional transfer of microRNA-loaded exosomes from T cells to antigen-presenting cells. *Nat Commun* 2: 282. [[PubMed](#)]
19. Valadi H, Ekström K, Bossios A, Sjöstrand 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: 654-659. [[PubMed](#)]
20. Théry C (2011) Exosomes: secreted vesicles and intercellular communications. *F1000 Biol Rep* 3: 15. [[PubMed](#)]
21. Parolini I, Federici C, Raggi C, Lugini L, Palleschi S, et al. (2009) Microenvironmental pH is a key factor for exosome traffic in tumor cells. *J Biol Chem* 284: 34211-34222. [[PubMed](#)]
22. Segura E, Guérin C, Hogg N, Amigorena S, Théry C (2007) CD8+ Dendritic Cells Use LFA-1 to Capture MHC-Peptide Complexes from Exosomes in vivo. *J Immunol* 179: 1489-1496. [[PubMed](#)]
23. Fitzner D, Schnaars M, van Rossum D, Krishnamoorthy G, Dibaj P, et al. (2011) Selective transfer of exosomes from oligodendrocytes to microglia by macropinocytosis. *J Cell Sci* 124: 447-458. [[PubMed](#)]
24. Montecalvo A, Larregina AT, Shufesky WJ, Stolz DB, Sullivan ML, et al. (2011) Mechanism of transfer of functional microRNAs between mouse dendritic cells via exosomes. *Blood* 119: 756-766. [[PubMed](#)]
25. Taylor DD, Bender DP, Gerçel-Taylor C, Stanson J, Whiteside TL (2001) Modulation of TcR/CD3- chain expression by a circulating factor derived from ovarian cancer patients. *Br J Cancer* 84: 1624-1629. [[PubMed](#)]
26. Clayton A, Mitchell JP, Court J, Mason MD, Tabi Z (2007) Human Tumor-Derived Exosomes Selectively Impair Lymphocyte Responses to Interleukin-2. *Cancer Res* 67: 7458-7466. [[PubMed](#)]
27. Iero M, Valenti R, Huber V, Filipazzi P, Parmiani G, et al. (2008) Tumour-released exosomes and their implications in cancer immunity. *Cell Death Differ* 15: 80-88. [[PubMed](#)]
28. Skog J, Würdinger T, van Rijn S, Meijer DH, Gainche L (2008) Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol* 10: 1470-1476. [[PubMed](#)]
29. Zomer A, Vendrig T, Hopmans ES, van Eijndhoven M, Middeldorp JM, et al. (2010) Exosomes Fit to deliver small RNA. *Commun Integr Biol* 3: 447-450. [[PubMed](#)]
30. Gesierich S, Berezovskiy I, Ryschich E, Zöller M (2006) Systemic Induction of the Angiogenesis Switch by the Tetraspanin D6.1A/CO-029. *Cancer Res* 66: 7083-7094. [[PubMed](#)]
31. Webber J, Steadman R, Mason MD, Tabi Z, Clayton A (2010) Cancer Exosomes Trigger Fibroblast to Myofibroblast Differentiation. *Cancer Res* 70: 9621-9630. [[PubMed](#)]
32. Thakur BK, Zhang H, Becker A, Matei I, Huang Y, et al. (2014) Double-stranded DNA in exosomes: a novel biomarker in cancer detection. *Cell Res* 24: 766-769. [[PubMed](#)]
33. Sukirtha R, Manasa PK, Joe AJ, Kamalakkannan S, Thangam R, et al. (2012) Cytotoxic effect of Green synthesized silver nanoparticles using *Melia azedarach* against in vitro HeLa cell lines and lymphoma mice model. *Process Biochem* 47: 273-279.
34. Bharti B, Mishra R (2011) Isoforms of Pax5 and co-regulation of T- and B-cells associated genes influence phenotypic traits of ascetic cells causing Dalton's lymphoma. *BBA-Mol Cell Res* 1813: 2071-2078. [[PubMed](#)]
35. Ramnath V, Rekha PS, Kuttan G, Kuttan R (2009) Regulation of Caspase-3 and Bcl-2 Expression in Dalton's Lymphoma Ascites Cells by Abrin. *J Evid Based Complementary Altern* 6: 233-238. [[PubMed](#)]
36. Evan GI, Vousden KH (2001) Proliferation, cell cycle and apoptosis in cancer. *Nature* 411: 342-348. [[PubMed](#)]