UM-EVs involved in tumor progression

Subjects: Oncology
Contributor: Thupten Tsering

Extracellular vesicles (EVs) carry molecules derived from donor cells and are able to alter the properties of recipient cells. They are important players during the genesis and progression of tumors. Uveal melanoma (UM) is the most common primary intraocular tumor in adults and is associated with a high rate of metastasis, primarily to the liver. However, the mechanisms underlying this process are poorly understood. In the present study, we analyzed the oncogenic potential of UM-derived EVs and their protein signature. We isolated and characterized EVs from five UM cell lines and from normal choroidal melanocytes (NCMs). BRCA1-deficient fibroblasts (Fibro-BKO) were exposed to the EVs and analyzed for their growth in vitro and their reprograming potential in vivo following inoculation into NOD-SCID mice. Mass spectrometry of proteins from UM-EVs and NCM-EVs was performed to determine a protein signature that could elucidate potential key players in UM progression. In-depth analyses showed the presence of exosomal markers, and proteins involved in cell-cell and focal adhesion, endocytosis, and PI3K-Akt signaling pathway. Notably, we observed high expression levels of HSP90, HSP70 and integrin V in UM-EVs. Our data bring new evidence on the involvement of UM-EVs in cancer progression and metastasis.

Keywords: Uveal melanoma ; extracellular vesicles ; liver metastasis ; liquid biopsy ; mass spectrometry

1. Introduction

Uveal Melanoma (UM) is the most common primary intraocular tumor in adults [1][2], and the second most common type of melanoma. It develops within the uveal tract of the eye, most frequently in the choroid [3][4]. Although there has been tremendous progress in understanding the genetic landscape [5][6][7][8], diagnosis [9][10][11], and treatment [12][13] of UM, the overall survival rate has not changed in the last three decades [14]. Its annual incidence is estimated at 3.75 and 5.2 cases per million individuals in Canada and the United States, respectively [15][16], while in Europe it varies according to latitude (2–8 cases per million) [17]. While the rate of UM occurrence is relatively low, approximately 50% of patients develop metastasis, primarily to the liver (90%) [18]. The 1-year survival rate of UM patients dramatically drops to 15% once it metastasizes [19] due to the absence of effective treatments and the high tumor burden at the time of detection [20]. While metastases are rarely detected at UM primary diagnosis, evidence has shown that circulating tumor cells can be found at diagnosis, suggesting that systemic involvement is an early phenomenon [21]. Moreover, the mechanisms underlying this process are not well understood. This implies that current clinical surveillance tools are not sensitive enough to detect premetastatic stages thereby underscoring the need for better and more sensitive biomarkers to complement and validate existing clinical surveillance. Extracellular vesicles (EVs) have been shown to harbor selective biomarkers in various cancers and to provide valuable clinical information [22]. However, the role of EVs as biomarkers and mediators of metastasis has not been widely explored in UM.

EVs are nanoparticles emitted under physiological and pathological conditions such as cancer. They are highly heterogeneous based on their size, shape and subcellular origin [23]. Minimal information for the study of EVs have now been standardized [24]; these include and are not limited to the size, floating density, as well as the presence of classical markers such as tetraspanins, annexins, Alix, and heat shock proteins (HSPs) [25][26]. The underlying molecular mechanism involved in EV formation, delivery of cargo inside EVs and ultimately in their release is still not clear [26][27]. In contrast, their uptake by target cells has been shown to occur via numerous pathways (i.e., endocytosis, micropinocytosis, phagocytosis) [28][29]. EVs are loaded with RNA [30][31], DNA [32], lipid [33][34] and proteins [35][36], and play a vital role in intercellular communications [37][38]. Notably, cancer-derived EVs promote cell proliferation, migration, invasion, angiogenesis and metastases [39][40][41].

A growing body of evidence proposes that EV cargo could be used as circulating biomarkers in liquid biopsy-based platform, particularly in the context of cancer. miRNA profiling of UM-derived exosomes has been performed [42][43]. In addition, the proteome profile of UM secretome and that of UM-derived EVs have also been reported [44][45]. However, none has addressed protein differential expression between healthy- and UM-derived EVs.

We performed this study to investigate the effects of EVs derived from UM cell lines on the behaviour of target cells, and to compare the protein contents in EVs derived from UM cells and normal choroidal melanocytes (NCMs). We have previously demonstrated that blood-derived EVs from patients with ocular and cutaneous melanoma are uptaken by and reprogram single oncosuppressor-mutated (SOM) cells into malignant cells [46][47][48]. Here, we showed that as opposed to NCM-EVs, UM-EVs increased the proliferation of target SOM cells such as BRCA1-deficient fibroblasts (Fibro-BKO) and induced their malignant transformation. In addition, proteomic analyses showed that UM-derived EVs were enriched in proteins involved in cell-cell and focal adhesion, endocytosis, and metastatic niche organization. Altogether, these data shed light on the role of EVs in driving cancer progression, and their potential use in a liquid biopsy platform to monitor patients affected by UM.

2. Discussion

UM is the only malignancy in which diagnosis is made by clinical examination and generally without a biopsy. Unfortunately, the very limited number of metastatic UM cases that are deemed appropriate for surgical resection limits the possibility to obtain surgical samples that can be analyzed to understand the metastatic process. Given the high mortality rate, the asymptomatic nature and the lack of monitoring biomarkers, a liquid biopsy platform would be extremely valuable in the diagnosis and treatment of UM. Attempts have been made to profile miRNA contents of UM-isolated exosomes [42][43], as well as the proteome from both UM secretome and UM-derived EVs [44][45]. However, research focusing on reliable and clinically valuable metastatic biomarkers in UM has remained limited, and an in-depth analysis of the protein composition of EV cargo in UM is virtually not available. Furthermore, studies addressing the differential expression pattern of EV cargo proteins between normal melanocytes and melanoma cells have been neither done nor published. It was already known that circulating EV levels increase with advancing stages of cancer, suggesting potential roles in cancer progression and invasion [49]. Furthermore, we previously reported that EVs from different malignancies carry oncogenic factors that trigger malignant transformation in target cells [50]. In the present study, we wanted to verify whether EVs isolated from UM cell lines would trigger malignant transformation of Fibro BKO. Moreover, we wanted to perform a label-free LC-MS/MS analyses on proteins isolated from EVs derived from both UM cells and NCMs to determine potential factors that may underlay the observed biological effects and to apply the findings as a base for a liquid biopsy platform.

Considering that UM arises from melanocytes of the uveal tract and due to the lack of a commercial source of normal uveal melanocytes we established a control line for comparative analyses isolating NCMs from the choroid tissue of donor eyes. The identity of NCMs was confirmed at both structural (cell shape) and phenotypical levels using a set of specific markers [51].

In our experiments we demonstrated that not only were UM-EVs efficiently internalized by Fibro BKO cells, but we also confirmed that these cells undergo dramatic changes after exposure to EVs as shown by increased proliferation, migration, invasion and acquisition of malignant characteristics. Moreover, factors carried by EVs belong to different molecular categories (i.e., DNA, mRNA, miRNA, proteins) and their roles in cancer biology have been extensively highlighted [52][53][54][55]. Recently, we provided evidence that cancer EVs actively transfer mutated cancer genes to target cells as well as a bulk of coding and non-coding RNAs acting as modulators of essential cellular pathways that impact cancer growth and progression. Herein, we decided to deepen these analyses by focusing on UM-EV protein cargo.

The proteomic analyses performed in this study confirmed the differential expression of several proteins involved in cancer cell growth, movement and adhesion, and metastatic niche remodeling. Although relatively rare, UM is a deadly disease mainly as a result of the high risk of metastases occurring primarily in the liver [56]. Our analysis revealed several typical proteins implicated in the establishment of premetastatic niche that were differently expressed in the EVs from UM cells compared to those from NCMs. It has previously been reported that tumor-derived EVs expressing ITGαvβv are implicated in liver metastasis organotropism. We observed high integrin αV protein levels in all UM-EVs analyzed when compared to NCM-EVs. Concurrently, DAVID bioinformatic analysis demonstrated that key proteins involved in the ITGαvβv complex were statistically significant in our dataset ($p < 0.05$). In relation to the integrins present in our UM-EV samples, there was upregulation of various signal transduction molecules such as S100A. It has been demonstrated that when integrins carried in cancer EVs were internalized by target cells, they activate SRC phosphorylation and pro-inflammatory S100 gene expression. Further, EVs from melanoma were found to upregulate S100 proteins in recipient target organ cells resulting in vascular leakiness and promotion of metastasis [57]. Taken together, this suggests that UM-EVs promote a tumor induced inflammatory response and metastatic niche formation. Such data may provide insight into UM's remarkable tropism to the liver.

Additionally, the expression patterns of both HSP90 and ENO1 were found uniformly increased across all UM-EVs. HSP90 is a molecular chaperone reported to be of crucial importance in cancer cell growth and survival owing to its involvement in promoting the MAPK and P13K/AKT pathways [58][59]. In similar fashion, ENO1 is linked to the AKT signaling pathway, is involved in promoting gastric cancer cell proliferation and metastasis and serves as a potential biomarker for certain cancers [60][61]. The PI3K-AKT signaling pathway was also highlighted in our KEGG pathway analysis as our UM-EVs contain a number of proteins linked to this signaling cascade. Other proteins involved in the process of metastasis were also identified in a set of UM-EVs (i.e., hepatocyte growth factor receptor tyrosine kinase (MET, in MP41 UM-EVs and MP46 UM-EVs), tenascin C (TNC, in MEL270 UM-EVs and MEL285 UM-EVs), ephrin-B2 (EFNB2, in MEL285 UM-EVs)) [60][62][61]. However, their expression was not uniformly increased in EVs derived from all UM cells.

Notably, when we mined for proteins differentially expressed between primary and metastatic UM-EVs (MEL270 UM-EVs vs. OMM2.5 UM-EVs), we found that primary UM-derived EVs were enriched for proteins involved in the regulation of cell growth (i.e., PCNA and CDK1) and in metastatic organotropism (i.e., integrins, Coronin 1C and CD151) [63][64]. Coronin 1C is highly expressed in invasive human cancers and correlates positively with increased metastatic risk [63]. CD151 is a tetraspanin associated with tumor metastasis, and is correlated with poor prognosis, decreased overall survival and increased recurrence [64]. Similarly, HSPB1 [65] was also increased in EVs derived from MEL270. HSPB1 has been reported to play an important role in UM micrometastasis [65] and acts as switch between tumor dormancy and tumor progression in breast cancer [66]. In contrast, proteins transported by metastatic UM-EVs are involved in the maintenance of the metastatic niche, mainly ECM modeling and organization (i.e., collagens, ECM1 and matrix metalloproteases (i.e., MMP2)) [67][68][69]. Previously, we reported that collagen IV-conveyed signals are essential cues for liver metastasis in several tumor types including UM and identified mediators of collagen IV signaling as potential therapeutic targets in the management of hepatic metastases. In addition, ECM1 promotes migration and invasion by inducing EMT [67], and MMP-2 is recognized as a crucial contributor to liver metastasis.

Our proteomic analysis unravels other markers that could be valuable as diagnostic and prognostic tools (i.e., Nidogen1; NID1). NID1 is a basement membrane glycoprotein that is involved in ECM cellular interactions, cell migration and invasion, promotes melanoma metastasis, and is correlated with poor clinical outcomes. In our study, high levels of NID1 were found in EVs derived from OMM2.5 (metastatic) cells. Previously, NID1 has been proposed as a new biomarker for disease progression and therapeutic target in breast cancer and melanoma [70].

In this study, we used an in vivo model to test whether UM-EVs could promote tumorigenesis. As shown by our group previously, exposure of cancer patient-derived EVs to single-oncogene mutated cells (such as Fibro-BKO, HEK 293 and PTEN KO MCF) resulted in malignant transformation of the recipient cells and induction of tumors in vivo. Here we injected NOD-SCID mice with Fibro-BKO cells exposed to UM cell-derived EVs and found a similar effect: Fibro-BKO cells exposed to UM cell-derived EVs developed tumors in vivo, while those exposed to NCM-derived EVs did not. Our in vivo study provides evidence that EVs derived from UM cancer cells have the potential to promote tumorigenesis in primed cells.

The selective enrichment of metastatic factors and signaling pathway components in UM-derived EVs will contribute to our overall understanding of the regulatory networks involved in the establishment of the tumor microenvironment. This information will be helpful in elucidating the pathophysiological functions of tumor-derived EVs, and aid in the development of UM diagnostics and therapeutics. In light of the data shown here, further studies to assess the downstream pathways that are altered in recipient cells are needed. Furthermore, understanding the role EVs play in mediating pro-tumor and in particular pro-metastasis processes in target organs, such as the liver are needed. Finally, validation of protein signatures, and potential biomarkers, are needed in EVs isolated from UM patient blood.

3. Conclusions

Metastasis is rarely found during diagnosis of primary UM and many patients already have organ specific micrometastases by the time the ocular tumor is detected [71]. Moreover, CTCs have been detected at primary UM diagnosis, preceding the clinical detection of metastasis [72]. There remains an urgent need for tools that will aid in the screening and monitoring of tumor burden. As the molecular contents of EVs reflect their cellular origin, EVs derived from cancer patient plasma can prove vital to the understanding of tumor progression, metastatic risk and allow real time evaluations of therapeutic outcome. This ability renders them prone to be used in liquid biopsy for detection of cancer biomarkers[73] [74]. In the present study, we profiled the proteome of pure preparations of EVs in the context of UM and

characterized their behaviour. The next step is to perform a comparative proteome profiling of EVs derived from both healthy individuals and from patients presenting with either uveal nevi or melanoma, with the ultimate goal of developing a non-invasive method to detect UM metastasis with high sensitivity and specificity.

References

1. Jovanovic, P.; Mihajlovic, M.; Djordjevic-Jocic, J.; Vlajkovic, S.; Cekic, S.; Stefanovic, V. Ocular melanoma: An overview of the current status. Int. J. Clin. Exp. Pathol. 2013, 6, 1230–1244.
2. Jager, M.J.; Brouwer, N.J.; Esmaeli, B. The Cancer Genome Atlas Project: An Integrated Molecular View of Uveal Melanoma. Ophthalmology 2018, 125, 1139–1142, doi:10.1016/j.ophtha.2018.03.011.
3. Ragusa, M.; Barbagallo, C.; Statello, L.; Caltabiano, R.; Russo, A.; Puzzo, L.; Avitabile, T.; Longo, A.; Toro, M.D.; Barbagallo, D.; et al. miRNA profiling in vitreous humor, vitreal exosomes and serum from uveal melanoma patients: Pathological and diagnostic implications. Cancer Biol. Ther. 2015, 16, 1387–1396, doi:10.1080/15384047.2015.1046021.
4. Krantz, B.A.; Dave, N.; Komatsubara, K.M.; Marr, B.P.; Carvajal, R.D. Uveal melanoma: Epidemiology, etiology, and treatment of primary disease. Clin. Ophthalmol. 2017, 11, 279–289, doi:10.2147/OPTH.S89591.
5. Field, M.G.; Harbour, J.W. Recent developments in prognostic and predictive testing in uveal melanoma. Curr. Opin. Ophthalmol. 2014, 25, 234–239, doi:10.1097/ICU.0000000000000051.
6. Kivelä, T.; Kujala, E. Prognostication in eye cancer: The latest tumor, node, metastasis classification and beyond. Eye 2013, 27, 243–252, doi:10.1038/eye.2012.256.
7. Coupland, S.E.; Lake, S.L.; Zeschnigk, M.; Damato, B.E. Molecular pathology of uveal melanoma. Eye 2013, 27, 230–242, doi:10.1038/eye.2012.255.
8. Harbour, J.W.; Chao, D.L. A molecular revolution in uveal melanoma: Implications for patient care and targeted therapy. Ophthalmology 2014, 121, 1281–1288, doi:10.1016/j.ophtha.2013.12.014.
9. Tarlan, B.; Kıratlı, H. Uveal Melanoma: Current Trends in Diagnosis and Management. Turk. J. Ophthalmol. 2016, 46, 123–137, doi:10.4274/tjo.37431.
10. Shields, J.A.; McDonald, P.R. Improvements in the Diagnosis of Posterior Uveal Melanomas. JAMA Ophthalmol. 1974, 91, 259–264, doi:10.1001/archopht.1974.03900060269004.
11. Rennie, I.G. Things that go bump in the light. The differential diagnosis of posterior uveal melanomas. Eye 2002, 16, 325–346, doi:10.1038/sj.eye.6700117.
12. Yang, J.; Manson, D.K.; Marr, B.P.; Carvajal, R.D. Treatment of uveal melanoma: Where are we now? Ther. Adv. Med. Oncol. 2018, 10, 1758834018757175, doi:10.1177/1758834018757175.
13. Carvajal, R.D.; Schwartz, G.K.; Tezel, T.; Marr, B.; Francis, J.H.; Nathan, P.D. Metastatic disease from uveal melanoma: Treatment options and future prospects. Br. J. Ophthalmol. 2017, 101, 38–44, doi:10.1136/bjophthalmol-2016-309034.
14. Papastefanou, V.P.; Cohen, V.M.L. Uveal melanoma. J. Skin Cancer 2011, 2011, 573974, doi:10.1155/2011/573974.
15. Ghazawi, F.M.; Darwich, R.; Le, M.; Rahme, E.; Zubarev, A.; Moreau, L.; Burnier, J.V.; Sasseville, D.; Burnier, M.N.; Litvinov, I.V. Uveal melanoma incidence trends in Canada: A national comprehensive population-based study. Br. J. Ophthalmol. 2019, 103, 1872–1876, doi:10.1136/bjophthalmol-2018-312966.
16. Aronow, M.E.; Topham, A.K.; Singh, A.D. Uveal Melanoma: 5-Year Update on Incidence, Treatment, and Survival (SEER 1973-2013). Ocul. Oncol. Pathol. 2018, 4, 145–151, doi:10.1159/000480640.
17. Virgili, G.; Gatta, G.; Ciccolallo, L.; Capocaccia, R.; Biggeri, A.; Crocetti, E.; Lutz, J.-M.; Paci, E.; Group, =EUROCARE Working Incidence of uveal melanoma in Europe. Ophthalmology 2007, 114, 2309–2315, doi:10.1016/j.ophtha.2007.01.032.
18. Kaliki, S.; Shields, C.L.; Shields, J.A. Uveal melanoma: Estimating prognosis. Indian J. Ophthalmol. 2015, 63, 93–102, doi:10.4103/0301-4738.154367.
19. Postow, M.A.; Kuk, D.; Bogatch, K.; Carvajal, R.D. Assessment of overall survival from time of metastastasis in mucosal, uveal, and cutaneous melanoma. J. Clin. Oncol. 2014, 32, 9074, doi:10.1200/jco.2014.32.15_suppl.9074.
20. Song, J.; Merbs, S.L.; Sokoll, L.J.; Chan, D.W.; Zhang, Z. A multiplex immunoassay of serum biomarkers for the detection of uveal melanoma. Clin. Proteom. 2019, 16, 10, doi:10.1186/s12014-019-9230-8.
21. Callejo, S.A.; Antecka, E.; Blanco, P.L.; Edelstein, C.; Burnier, M.N. Identification of circulating malignant cells and its correlation with prognostic factors and treatment in uveal melanoma. A prospective longitudinal study. Eye 2007, 21, 752–759, doi:10.1038/sj.eye.6702322.

22. Surman, M.; Stępień, E.; Przybyło, M. Melanoma-Derived Extracellular Vesicles: Focus on Their Proteome. Proteomes 2019, 7, 21.

23. van Niel, G.; D'Angelo, G.; Raposo, G. Shedding light on the cell biology of extracellular vesicles. Nat. Rev. Mol. Cell Biol. 2018, 19, 213.

24. Théry, C.; Witwer, K.W.; Aikawa, E.; Alcaraz, M.J.; Anderson, J.D.; Andriantsitohaina, R.; Antoniou, A.; Arab, T.; Archer, F.; Atkin-Smith, G.K.; et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): A position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J. Extracell. Vesicles 2018, 7, 1535750, doi:10.1080/20013078.2018.1535750.

25. Simpson, R.J.; Jensen, S.S.; Lim, J.W.E. Proteomic profiling of exosomes: Current perspectives. Proteomics 2008, 8, 4083–4099, doi:10.1002/pmic.200800109.

26. Jeppesen, D.K.; Fenix, A.M.; Franklin, J.L.; Higginbotham, J.N.; Zhang, Q.; Zimmerman, L.J.; Liebler, D.C.; Ping, J.; Liu, Q.; Evans, R.; et al. Reassessment of Exosome Composition. Cell 2019, 177, 428–445.e18, doi:10.1016/j.cell.2019.02.029.

27. Lee, T.H.; Chennakrishnaiah, S.; Meehan, B.; Montermini, L.; Garnier, D.; D'Asti, E.; Hou, W.; Magnus, N.; Gayden, T.; Jabado, N.; et al. Barriers to horizontal cell transformation by extracellular vesicles containing oncogenic H-ras. Oncotarget 2016, 7, 51991–52002, doi:10.18632/oncotarget.10627.

28. Horibe, S.; Tanahashi, T.; Kawauchi, S.; Murakami, Y.; Rikitake, Y. Mechanism of recipient cell-dependent differences in exosome uptake. BMC Cancer 2018, 18, 47, doi:10.1186/s12885-017-3958-1.

29. Feng, D.; Zhao, W.-L.; Ye, Y.-Y.; Bai, X.-C.; Liu, R.-Q.; Chang, L.-F.; Zhou, Q.; Sui, S.-F. Cellular Internalization of Exosomes Occurs Through Phagocytosis. Traffic 2010, 11, 675–687, doi:10.1111/j.1600-0854.2010.01041.x.

30. Bellingham, S.A.; Coleman, B.M.; Hill, A.F. Small RNA deep sequencing reveals a distinct miRNA signature released in exosomes from prion-infected neuronal cells. Nucleic Acids Res. 2012, 40, 10937–10949, doi:10.1093/nar/gks832.

31. Di Vizio, D.; Morello, M.; Dudley, A.C.; Schow, P.W.; Adam, R.M.; Morley, S.; Mulholland, D.; Rotinen, M.; Hager, M.H.; Insabato, L.; et al. Large oncosomes in human prostate cancer tissues and in the circulation of mice with metastatic disease. Am. J. Pathol. 2012, 181, 1573–1584, doi:10.1016/j.ajpath.2012.07.030.

32. Kahlert, C.; Melo, S.A.; Protopopov, A.; Tang, J.; Seth, S.; Koch, O.; Zhang, J.; Weitz, J.; Chin, L.; Futreal, A.; et al. Identification of doublestranded genomic dna spanning all chromosomes with mutated KRAS and P53 DNA in the serum exosomes of patients with pancreatic cancer. J. Biol. Chem. 2014, 289, 3869–3875, doi:10.1074/jbc.C113.532267.

33. Brzozowski, J.S.; Jankowski, H.; Bond, D.R.; McCague, S.B.; Munro, B.R.; Predebon, M.J.; Scarlett, C.J.; Skelding, K.A.; Weidenhofer, J. Lipidomic profiling of extracellular vesicles derived from prostate and prostate cancer cell lines. Lipids Health Dis. 2018, 17, 211, doi:10.1186/s12944-018-0854-x.

34. Skotland, T.; Sandvig, K.; Llorente, A. Lipids in exosomes: Current knowledge and the way forward. Prog. Lipid Res. 2017, 66, 30–41, doi:10.1016/j.plipres.2017.03.001.

35. Théry, C.; Boussac, M.; Véron, P.; Ricciardi-Castagnoli, P.; Raposo, G.; Garin, J.; Amigorena, S. Proteomic Analysis of Dendritic Cell-Derived Exosomes: A Secreted Subcellular Compartment Distinct from Apoptotic Vesicles. J. Immunol. 2001, 166, 7309–7318, doi:10.4049/jimmunol.166.12.7309.

36. Liang, B.; Peng, P.; Chen, S.; Li, L.; Zhang, M.; Cao, D.; Yang, J.; Li, H.; Gui, T.; Li, X.; et al. Characterization and proteomic analysis of ovarian cancer-derived exosomes. J. Proteom. 2013, 80, 171–182, doi:10.1016/j.jprot.2012.12.029.

37. Zaborowski, M.P.; Balaj, L.; Breakefield, X.O.; Lai, C.P. Extracellular Vesicles: Composition, Biological Relevance, and Methods of Study. Bioscience 2015, 65, 783–797, doi:10.1093/biosci/biv084.

38. Inamdar, S.; Nitiyanandan, R.; Rege, K. Emerging applications of exosomes in cancer therapeutics and diagnostics. Bioeng. Transl. Med. 2017, 2, 70–80, doi:10.1002/btm2.10059.

39. Al-Nedawi, K.; Meehan, B.; Micallef, J.; Lhotak, V.; May, L.; Guha, A.; Rak, J. Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. Nat. Cell Biol. 2008, 10, 619–624, doi:10.1038/ncb1725.

40. Aga, M.; Bentz, G.L.; Raffa, S.; Torrisi, M.R.; Kondo, S.; Wakisaka, N.; Yoshizaki, T.; Pagano, J.S.; Shackelford, J. Exosomal HIF1α supports invasive potential of nasopharyngeal carcinoma-associated LMP1-positive exosomes. Oncogene 2014, 33, 4613–4622, doi:10.1038/onc.2014.66.

41. Xu, J.; Liao, K.; Zhou, W. Exosomes Regulate the Transformation of Cancer Cells in Cancer Stem Cell Homeostasis. Stem Cells Int. 2018, 2018, 4837370, doi:10.1155/2018/4837370.

42. Kilic, E.; Smit, K.; van Poppelen, N.; Lunavat, T.; Derks, K.; Vaarwater, J.; Verdijk, R.; Mensink, H.; Lötvall, J.; de Klein, A. miRNA profiling of uveal melanoma exosomes as a metastatic risk biomarker. Acta Ophthalmol. 2017, 95, doi:10.1111/j.1755-3768.2017.03642.

43. Eldh, M.; Olofsson Bagge, R.; Lässer, C.; Svanvik, J.; Sjöstrand, M.; Mattsson, J.; Lindnér, P.; Choi, D.-S.; Gho, Y.S.; L ötvall, J. MicroRNA in exosomes isolated directly from the liver circulation in patients with metastatic uveal melanoma. BMC Cancer 2014, 14, 962, doi:10.1186/1471-2407-14-962.
44. Pardo, M.; García, Á.; Antrobus, R.; Blanco, M.J.; Dwek, R.A.; Zitzmann, N. Biomarker Discovery from Uveal Melanom a Secretomes: Identification of gp100 and Cathepsin D in Patient Serum. J. Proteome Res. 2007, 6, 2802–2811, doi:1 0.1021/pr070021t.
45. Angi, M.; Kalirai, H.; Prendergast, S.; Simpson, D.; Hammond, D.E.; Madigan, M.C.; Beynon, R.J.; Coupland, S.E. In-d epth proteomic profiling of the uveal melanoma secretome. Oncotarget 2016, 7, 49623–49635, doi:10.18632/oncotarge t.10418.
46. Abdouh, M.; Hamam, D.; Gao, Z.-H.; Arena, V.; Arena, M.; Arena, G.O. Exosomes isolated from cancer patients' sera tr ansfer malignant traits and confer the same phenotype of primary tumors to oncosuppressor-mutated cells. J. Exp. Cli n. Cancer Res. 2017, 36, 113, doi:10.1186/s13046-017-0587-0.
47. Hamam, D.; Abdouh, M.; Gao, Z.-H.; Arena, V.; Arena, M.; Arena, G.O. Transfer of malignant trait to BRCA1 deficient h uman fibroblasts following exposure to serum of cancer patients. J. Exp. Clin. Cancer Res. 2016, 35, 80, doi:10.1186/s 13046-016-0360-9.
48. Abdouh, M.; Gao, Z.H.; Arena, V.; Arena, M.; Burnier, M.N.; Arena, G.O. Oncosuppressor-Mutated Cells as a Liquid Bio psy Test for Cancer-Screening. Sci. Rep. 2019, 9, 2384, doi:10.1038/s41598-019-38736-y.
49. Zhang, X.; Yuan, X.; Shi, H.; Wu, L.; Qian, H.; Xu, W. Exosomes in cancer: Small particle, big player. J. Hematol. Onco l. 2015, 8, 83, doi:10.1186/s13045-015-0181-x.
50. Abdouh, M.; Floris, M.; Gao, Z.-H.; Arena, V.; Arena, M.; Arena, G.O. Colorectal cancer-derived extracellular vesicles in duce transformation of fibroblasts into colon carcinoma cells. J. Exp. Clin. Cancer Res. 2019, 38, 257, doi:10.1186/s13 046-019-1248-2.
51. Weidmann, C.; Pomerleau, J.; Trudel-vandal, L.; Landreville, S. Differential responses of choroidal melanocytes and uv eal melanoma cells to low oxygen conditions. Mol. Vis. 2017, 23, 103–115.
52. Skog, J.; Würdinger, T.; van Rijn, S.; Meijer, D.H.; Gainche, L.; Sena-Esteves, M.; Curry, W.T., Jr.; Carter, B.S.; Krichev sky, A.M.; Breakefield, X.O. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and pr ovide diagnostic biomarkers. Nat. Cell Biol. 2008, 10, 1470–1476, doi:10.1038/ncb1800.
53. Hood, J.L.; San Roman, S.; Wickline, S.A. Exosomes released by melanoma cells prepare sentinel lymph nodes for tu mor metastasis. Cancer Res. 2011, 71, 3792–3801, doi:10.1158/0008-5472.CAN-10-4455.
54. Fujita, Y.; Yoshioka, Y.; Ochiya, T. Extracellular vesicle transfer of cancer pathogenic components. Cancer Sci. 2016, 10 7, 385–390, doi:10.1111/cas.12896.
55. Valadi, H.; Ekström, K.; Bossios, A.; Sjöstrand, M.; Lee, J.J.; Lötvall, J.O. Exosome-mediated transfer of mRNAs and m icroRNAs is a novel mechanism of genetic exchange between cells. Nat. Cell Biol. 2007, 9, 654–659, doi:10.1038/ncb1 596.
56. Group Collaborative Ocular Melanoma Study Development of Metastatic Disease After Enrollment in the COMS Trials f or Treatment of Choroidal Melanoma: Collaborative Ocular Melanoma Study Group Report No. 26. JAMA Ophthalmol. 2005, 123, 1639–1643, doi:10.1001/archopht.123.12.1639.
57. Peinado, H. Melanoma exosomes educate bone marrow progenitor cells. Nat. Med. 2013, 18, 883–891, doi:10.1038/n m.2753.Melanoma.
58. Rong, B.; Yang, S. Molecular mechanism and targeted therapy of Hsp90 involved in lung cancer: New discoveries and developments (Review). Int. J. Oncol. 2018, 52, 321–336, doi:10.3892/ijo.2017.4214.
59. Mielczarek-Lewandowska, A.; Hartman, M.L.; Czyz, M. Inhibitors of HSP90 in melanoma. Apoptosis 2019, 25, 12–28, d oi:10.1007/s10495-019-01577-1.
60. Surriga, O.; Rajasekhar, V.K.; Ambrosini, G.; Dogan, Y.; Huang, R.; Schwartz, G.K. Crizotinib, a c-Met Inhibitor, Prevent s Metastasis in a Metastatic Uveal Melanoma Model. Mol. Cancer Ther. 2013, 12, 2817–2826, doi:10.1158/1535-7163. MCT-13-0499.
61. Kääriäinen, E.; Nummela, P.; Soikkeli, J.; Yin, M.; Lukk, M.; Jahkola, T.; Virolainen, S.; Ora, A.; Ukkonen, E.; Saksela, O.; et al. Switch to an invasive growth phase in melanoma is associated with tenascin-C, fibronectin, and procollagen-I forming specific channel structures for invasion. J. Pathol. 2006, 210, 181–191, doi:10.1002/path.2045.
62. Cheng, H.; Chua, V.; Liao, C.; Purwin, T.J.; Terai, M.; Kageyama, K.; Davies, M.A.; Sato, T.; Aplin, A.E. Co-targeting HG F/cMET Signaling with MEK Inhibitors in Metastatic Uveal Melanoma. Mol. Cancer Ther. 2017, 16, 516–528, doi:10.115 8/1535-7163.MCT-16-0552.

63. Castagnino, A.; Castro-Castro, A.; Irondelle, M.; Guichard, A.; Lodillinsky, C.; Fuhrmann, L.; Vacher, S.; Agüera-González, S.; Zagryazhskaya-Masson, A.; Romao, M.; et al. Coronin 1C promotes triple-negative breast cancer invasiveness through regulation of MT1-MMP traffic and invadopodia function. Oncogene 2018, 37, 6425–6441, doi:10.1038/s41388-018-0422-x.

64. Ke, A.W.; Shi, G.M.; Zhou, J.; Wu, F.Z.; Ding, Z. Bin; Hu, M.Y.; Xu, Y.; Song, Z.J.; Wang, Z.J.; Wu, J.C.; et al. Role of overexpression of CD151 and/or c-Met in predicting prognosis of hepatocellular carcinoma. Hepatology 2009, 49, 491–503, doi:10.1002/hep.22639.

65. Crabb, J.W.; Hu, B.; Crabb, J.S.; Triozzi, P.; Saunthararajah, Y.; Tubbs, R.; Singh, A.D. iTRAQ quantitative proteomic comparison of metastatic and non-metastatic uveal melanoma tumors. PLoS ONE 2015, 10, e0135543, doi:10.1371/journal.pone.0135543.

66. Lee, Y.-J.; Lee, H.-J.; Choi, S.; Jin, Y.B.; An, H.J.; Kang, J.-H.; Yoon, S.S.; Lee, Y.-S. Soluble HSPB1 regulates VEGF-mediated angiogenesis through their direct interaction. Angiogenesis 2012, 15, 229–242, doi:10.1007/s10456-012-9255-3.

67. Chen, H.; Jia, W.; Li, J. ECM1 promotes migration and invasion of hepatocellular carcinoma by inducing epithelial-mesenchymal transition. World J. Surg. Oncol. 2016, 14, 195, doi:10.1186/s12957-016-0952-z.

68. Liu, Y.; Zhang, J.; Chen, Y.; Sohel, H.; Ke, X.; Chen, J.; Li, Y.X. The correlation and role analysis of COL4A1 and COL4A2 in hepatocarcinogenesis. Aging (Albany NY) 2020, 12, 204–223, doi:10.18632/aging.1026102610.

69. Wu, Y.H.; Chang, T.H.; Huang, Y.F.; Huang, H.D.; Chou, C.Y. COL11A1 promotes tumor progression and predicts poor clinical outcome in ovarian cancer. Br. Dent. J. 2014, 217, 3432–3440, doi:10.1038/onc.2013.307.

70. Alečković, M.; Wei, Y.; LeRoy, G.; Sidoli, S.; Liu, D.D.; Garcia, B.A.; Kang, Y. Identification of Nidogen 1 as a lung metastasis protein through secretome analysis. Genes Dev. 2017, 31, 1439–1455, doi:10.1101/gad.301937.117.

71. Damato, B. Does ocular treatment of uveal melanoma influence survival? Br. J. Cancer 2010, 103, 285–290, doi:10.1038/sj.bjc.6605765.

72. Anand, K.; Roszik, J.; Gombos, D.; Upshaw, J.; Sarli, V.; Meas, S.; Lucci, A.; Hall, C.; Patel, S. Pilot Study of Circulating Tumor Cells in Early-Stage and Metastatic Uveal Melanoma. Cancers 2019, 11, 856, doi:10.3390/cancers11060856.

73. Eguchi, A.; Kostallari, E.; Feldstein, A.E.; Shah, V.H. Extracellular vesicles, the liquid biopsy of the future. J. Hepatol. 2019, 70, 1292–1294, doi:10.1016/j.jhep.2019.01.030.

74. Zhao, Z.; Fan, J.; Hsu, Y.-M.S.; Lyon, C.J.; Ning, B.; Hu, T.Y. Extracellular vesicles as cancer liquid biopsies: From discovery, validation, to clinical application. Lab Chip 2019, 19, 1114–1140, doi:10.1039/C8LC01123K.
