EXOSOMES AND CANCER

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White Paper No 101
September, 2013

Exosomes are small vesicles which are ejected from cells and contain RNA and other cell fragments. Recent work has demonstrated their usefulness in cancer diagnostics and prognostics. We examine some of these applications.

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1 INTRODUCTION[1]

Exosomes are small vesicles released by a cell and containing various RNA and proteins, often specific to the cell and the cell state. They are released into the extracellular matrix and may then find their way into blood, urine, lymph and other systems from which they can be extracted and examined. These exosomes have recently been better understood and techniques to gather and analyze them have been perfected.

Cell collect proteins, RNA and others elements and pack them up in small packages and export them to the surface of the cell to be expelled. The expelled packages are called exosomes and they are in effect messengers of what is happening within the cell. Being able to read these messages allows one possibly to understand what is happening within the cell.

One of the recent but stage setting papers by Skog et al noted in 2008:

Glioblastoma tumour cells release microvesicles (exosomes) containing mRNA, miRNA and angiogenic proteins. These microvesicles are taken up by normal host cells, such as brain microvascular endothelial cells.

By incorporating an mRNA for a reporter protein into these microvesicles, we demonstrate that messages delivered by microvesicles are translated by recipient cells. These microvesicles are also enriched in angiogenic proteins and stimulate tubule formation by endothelial cells. Tumour-derived microvesicles therefore serve as a means of delivering genetic information and proteins to recipient cells in the tumour environment. Glioblastoma microvesicles also stimulated proliferation of a human glioma cell line, indicating a self-promoting aspect.

Messenger RNA mutant/variants and miRNAs characteristic of gliomas could be detected in serum microvesicles of glioblastoma patients. The tumour-specific EGFRvIII was detected in serum microvesicles from 7 out of 25 glioblastoma patients. Thus, tumour-derived microvesicles may provide diagnostic information and aid in therapeutic decisions for cancer patients through a blood test.

Thus one can use the RNAs detected by the transported exosomes to detect the cancer. This approach can be applied across a wide base of cancers. Namely prostate cancer may be detected via exosomes in the urine, melanoma in exosomes in the blood as well as ovarian cancers (see Taylor and Taylor).

We examine these exosomes as applied to several cancers and discuss their potential diagnostic and prognostic capabilities.

It should be noted that there is significant work being done in many areas to develop non-invasive means of testing for the presence of cancers as well as for the determination of

[1] I want to thank Dr. James McKiernan at Columbia University Medical Center for introducing me to these ideas and discussing them with me at length. All comments and observations are mine and do not necessarily reflect the opinions of Dr. McKiernan. I also met with the management of Exosomedx and what is discussed in this paper is based solely on public documents and not on what I was informed about.

prognostic measures. For example, the now classic PSA test is one who use had bloomed but who efficacy has been called into question despite its recognized ability to reduce prostate cancer mortality in cases where it is properly applied. We have discussed that at length in our work on Prostate Cancer Genomics. However, and this is a critical factor, the main driver has been the lack of high sensitivity and specificity. This measure is the area under the sensitivity-specificity curve. Ideally we would like to have a measure that has an area of 1.0. Total ambiguity occurs when the area is 0.5, namely pure guess work. Recent tests using exosomes on prostate cancer have shown increased ROC areas, namely better than PSA albeit not perfect. One must note that even prostate biopsies are not perfect.

We begin with a brief review of the exosome and then focus on two specific cancers; prostate and melanoma. We conclude with several observations.

2 EXOSOMES

We begin with a description of the exosomes. We use the paper by Keller et al as an excellent example of exosomes description.

2.1 EXOSOME STRUCTURES

First, we begin with a description of multi-vesicular bodies, MVB, those intracellular elements which are to be expressed in some manner outwards of the cell. Keller et al state:

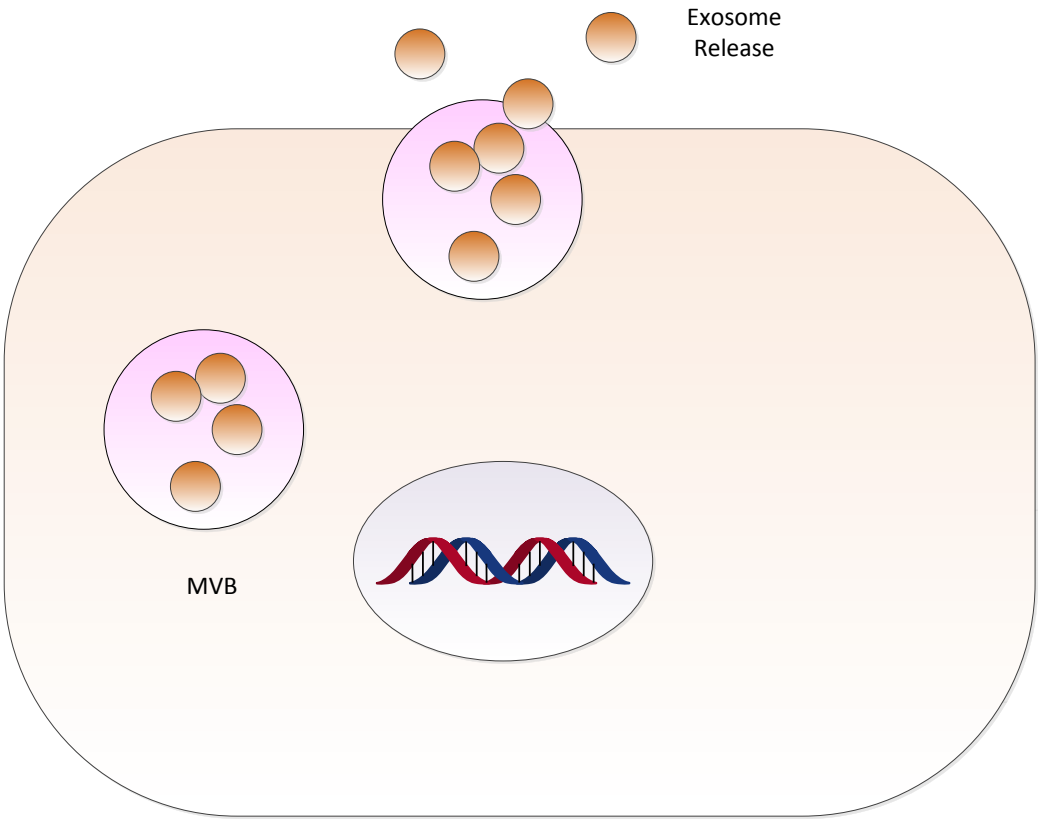
Fusion of multivesicular bodies (MVBs) with the plasma membrane and the subsequent release of their cargo represent another mechanism of exocytosis. Since the development of these membrane vesicles has an endocytic origin, this mechanism is a secretion process of the endosomal system. Other components of the endosomal system include endocytic vesicles, early endosomes, late endosomes and lysosomes.

Endocytic vesicles arise through clathrin- or non-clathrin-mediated endocytosis at the plasma membrane and are transported to early endosomes. Late endosomes develop from early endosomes by acidification, changes in their protein content and their tendency to fuse with vesicles or more generally with other membranes

They then continue to state:

MVBs can also fuse with the plasma membrane leading to the release of the internal vesicles into the extracellular space. The released vesicles are then called exosomes. Many cell types release exosomes via this mechanism including hematopoietic cells, reticulocytes, B- and T-lymphocytes, dendritic cells, mast cells, platelets, intestinal epithelial cells, astrocytes, neurons and tumor cells. Depending on their origin, exosomes have previously been named dexosomes (dendritic cell-derived exosomes) or texosomes (T-cell exosomes or tumor exosomes).

The following Figure is depictive of the exosomes.



Thus exosomes are encapsulated vesicles containing rejected molecules from the cell. These molecules, especially the RNA elements, may have significant diagnostic and prognostic value.

2.2 EXOSOMES AND SIGNALLING

Exosomes send information from within the cell to the outside and then in turn to other cells. This thus makes the exosome an interesting carrier as well as reporter of what is within the cell. As Rama et al state:

Exosome binding and uptake by target cells are also selective processes that involve various endocytic pathways and proteins from exosome donor and target cells, where exosomal tetraspanin complexes bind to selected ligands, which are also located in internalization-prone microdomains . Exosomal proteins, mRNA, and miRNA are functionally active and exosome binding/uptake can severely alter target cells, as demonstrated for T cell activation, immunosuppression, and conversion to a malignant phenotype

They conclude:

Finally, supporting the concept of a central role of tumor exosomes in metastasis, exosomal miRNA from a metastasizing tumor line, though not being oncogenic, preferentially regulates RNA, which contributes to establishing a premetastatic niche. Exosomes are discussed as a most potent gene delivery system.

Our findings support this hypothesis and suggest that competing tumor exosomes could well be a promising therapeutic option by preventing establishing a premetastatic niche. Beyond this, tailored exosomes might allow to rescind tumor exosome–induced host cell modulation.

The concept of exosomes as also a delivery system is somewhat novel and it is well worth the continued exploration of such concepts.

From Duijvesz et al we have:

Exosome shedding is a process with a wide range of important regulatory functions. Their discovery in sheep reticulocyte maturation gave rise to the idea that exosomes may function as a trash bin for unnecessary and redundant proteins and therefore could be an alternative pathway for lysosomal degradation.

Nevertheless, most attention has been paid to their role in the immune system. Functional experiments have shown that exosomes affect the immune system by expressing and processing antigens

First, exosomes are enriched with specific antigens, compared to whole-cell lysates.

Second, exosomes from antigen-presenting cells (APCs) contain large amounts of major histocompatibility complex (MHC) class I and II molecules.

When APC-derived exosomes are incubated with donor cells, MHC could be reexpressed in these cells. These results indicate that there is an exchange of membranes or membrane proteins between exosomes and cells and that, consequently, exosomes harbour a communicative function. Aside from the membrane transfer, exosome content such as proteins and RNAs can also be shuttled between cells through exosomes.

By transferring RNAs, exosomes are capable of transferring genetic information that can be translated into functional proteins in target cells [28]. In the field of cancer research, there is an ongoing debate regarding the exact role of exosomes as pro- or antitumour effectors.

Experiments in mice have shown that cancer derived exosomes can induce protective antitumour immune responses. It has been demonstrated that exosomes isolated from malignant effusions are an effective source of tumour antigens to be presented to CD8+ cytotoxic T cells

Thus the exosomes is a powerful tool in the arsenal to deal with cancer cells.

3 MELANOMA

Several authors have examined melanomas regarding the diagnosis and prognosis of melanoma. We briefly summarize their results here. These papers clearly demonstrate the role of exosomes from melanoma cells as communicators across the intercellular matrix resulting in metastatic growth, proliferation and spread.

3.1 MELANOCYTE TO MELANOCYTE TRANSMISSION

First the paper by Xiao et al states:

Our results indicate that melanoma-derived exosomes have unique gene expression signatures, miRNA and proteomics profiles compared to exosomes from normal melanocytes. To the best of our knowledge, this is the first in-depth screening of the whole transcriptome-miRNome proteome expression in melanoma exosomes. These results provide a starting point for future more in-depth studies of tumor-derived melanoma exosomes, which will aid our understanding of melanoma biogenesis and new drug-targets that may be translated into clinical applications, or as noninvasive biomarkers for melanoma.

They continue[2]:

Exosomes are small endosome-derived vesicles ranging in size from 40–100 nm in diameter that are actively secreted from cells through exocytosis, a process normally used for receptor discharge and intercellular cross-talk. Many types of cells have the capacity to release exosomes, including retinocytes, dendritic cells, B cells, T cells, mast cells, epithelial cells and tumor cells.

Secreted exosomes have been isolated and characterized in vitro from cultured cell lines, as well as in vivo in body fluids including blood, urine, saliva, amniotic fluid, and malignant pleural effusions. Exosome levels in blood and other body fluids increases with advancing stage of cancer. Exosomes have pleiotropic biological functions, including regulation of immune responses, antigen presentation, intercellular communication, tumor proliferation, and the transfer of RNA and proteins between cells.

Tumor exosomes have intact and functional mRNAs, small RNAs, and proteins that can alter the cellular environment to favor tumor growth.

Exosome mRNA can also produce protein in the presence of functional protein machinery. MicroRNAs (miRNAs) are short RNAs (21–23 nucleotides) that bind to the 39 untranslated regions of target genes causing translational repression of the target gene, and stimulating rapid degradation of the target transcript. miRNAs represent a new species of genetic regulator, controlling the levels of potentially large numbers of proteins

[2] It should be noted that Xiao et al have an excellent description of their extraction methods and their determination techniques.

Xiao et al conclude:

1. Normal Melanocytes Acquire Invasiveness through Uptaking of Melanoma Cell-derived Exosomes

2. Gene Expression Changes in Normal Melanocytes after Uptake of Melanoma Cell-derived Exosomes

These are powerful conclusions since we can not only understand certain diagnostic and prognostic information but also understand the intercellular communications leading to the expansion of melanoma.

3.2 BONE MARROW SPREAD

In the paper by Peinado et al they discuss the metastatic spread of melanoma via an exosomes route as well. They conclude:

Exosomes from highly metastatic melanomas increased the metastatic behavior of primary tumors by permanently 'educating' bone marrow progenitors through the receptor tyrosine kinase MET. Melanoma-derived exosomes also induced vascular leakiness at pre-metastatic sites and reprogrammed bone marrow progenitors toward a pro-vasculogenic phenotype that was positive for c-Kit, the receptor tyrosine kinase Tie2 and Met. Reducing Met expression in exosomes diminished the pro-metastatic behavior of bone marrow cells….

Our data show that exosome production, transfer and education of bone marrow cells supports tumor growth and metastasis, has prognostic value and offers promise for new therapeutic directions in the metastatic process….

Although the membrane vesicles of cancer cells have been suggested to contribute to the horizontal propagation of oncoproteins and genetic material (RNA)5,8,13,14,40,48, our study is the first, to our knowledge, to show that transfer of the MET oncoprotein from tumor-derived exosomes to bone marrow progenitor cells promotes the metastatic process in vivo. Notably, we show that exosomes can alter the bone marrow in a durable manner; these results suggest that genetic or epigenetic changes could be involved in this phenomenon, as bone marrow cells retain the educated phenotype after engraftment into a new host.

Here we propose a new mechanism that controls metastatic progression through the crosstalk between tumor-derived exosomes and bone marrow progenitor cells. Collectively, our data identify exosome-mediated transfer of the oncoprotein MET as a key regulator of bone marrow education and mobilization and metastatic progression.

3.3 LYMPH NODE SPREAD

Finally in the paper by Hood et al discusses the exosomes as a lymph node metastatic element. They state:

In order to metastasize, tumor cells must manipulate their microenvironment to optimize conditions for deposition and growth both locally and at a distance. In accordance with the "seed and soil" hypothesis for example cancer stem cells or metastatic cells function as "seeds" and a particular organ microenvironment or niche serves as the "soil". Potential sites for remote tumor implantation might thus be prepared well ahead of actual metastasis. For specific cancers such a metastatic melanoma, the process of metastasis involves lymphatic dissemination although the precise role lymph nodes play in supporting this process is not defined.

In one hypothesis melanoma cells undergo simultaneous hematogenous and lymphatic spread and the presence of tumor cells in sentinel or regional nodes is merely indicative of metastasis. Alternatively, sentinel or regional nodes play an active role in the progression of melanoma metastasis. The observation that regional lymph nodes downstream of melanomas undergo reactive lymphangiogenesis prior to metastasis (6) suggest that melanoma metastasis is facilitated by preparation of a pre-metastatic niche within lymph nodes. This process is believed to be mediated by tumor secretion of paracrine angiogenic growth factors. In this report we demonstrate an adjunctive and highly efficient model of pre-metastatic niche formation in regional lymph nodes through the local actions of melanoma exosomes.

Exosomes are naturally occurring biological nanovesicles (~ 30-100 nm) that are formed by the inward budding of multivesicular bodies (MVBs), as a component of the endocytic pathway. They are generated constitutively and released into the tumor microenvironment and circulation via fusion of multivesicular bodies with the tumor cell plasma membrane. The nanoscale size of exosomes facilitates their penetration and interaction with local tumor cells as well as with cell types that are distant to an advancing tumor cell front. This may result in tumor immune evasion by direct suppression of T cell activation and induction of apoptosis, suppression of the anti-tumor activity of natural killer cells and other mechanisms.

They continue:

Given the predilection of melanoma to metastasize via lymphatics, we hypothesized that melanoma exosomes travel to sentinel lymph nodes. To the best of our knowledge this is the first study of its kind to assess lymphatic trafficking of any type of exosome. Therefore, we constructed a control liposome to determine whether trafficking patterns would differ between melanoma exosomes versus inert bland nanovesicles lacking protein, mRNA, miRNA or other complex molecular variables found in exosomes.

Finally, the presence of melanoma exosomes in lymph nodes leads to induction of angiogenic growth factors necessary for melanoma growth. VEGF-B expression is increased by metastatic melanoma cells (38) and maintains survival of neovasculature (39). Increased HIF1- α expression by melanoma cells contributes to malignancy (40), increased VEGF expression (41) and poor prognosis.

4 PROSTATE CANCER

There has been a great deal of debate as to how best to monitor men for potential prostate cancer. In addition there is a significant discussion on the idea of watchful waiting, namely doing nothing until one "has to". The problem with the latter issue is that we have no idea what PCa is indolent and which is aggressive. Perhaps we can use exosomes as a step in solving this uncertainty.

4.1 PCA AND OTHER MEASURES

From the paper by Duijvesz et al; we have the following Table (modified) which summarizes the different vesicles, their size and function. The first class is the exosomes which we have examined herein. There are many other classes of vesicles which are summarized below.

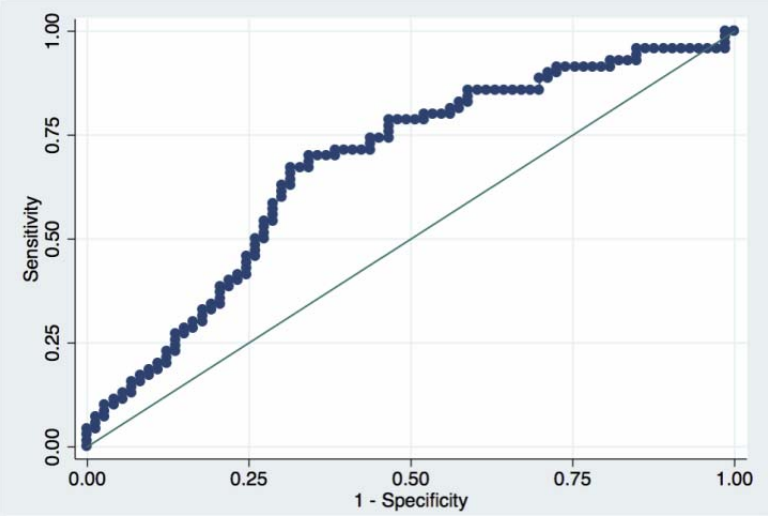
Vesicle	*Size*	*Known Protein Markers*	*RNA Markers*	*Synthesis Pathway*	*Function*
Exosomes	50–150	CD9, CD63, CD81, CD82,annexins, and RAB proteins	PCA3, TMPRSS2:ERG	Merocrine	Antigen presentation, immune regulatory, and metastatic activity
Prostasomes	50–500	CD13, CD46, CD55, CD59, annexins, and RAB proteins	–	Merocrine and apocrine	Immunosuppressive and sperm cell motility improving
Oncosomes	50–500	Signal transduction proteins	DIAPH3	Apocrine	ND
Microvesicles	100–1000	Integrins, selectins, and CD40 ligand	EGFRvIII	Apocrine	Pro-coagulation and anticoagulation
Ectosomes (microparticles)	50–1000	CR1 and proteolytic enzymes	–	Apocrine	Procoagulation and anticoagulation

4.2 IMPROVED DIAGNOSTIC AND PROGNOSTIC MEASURES

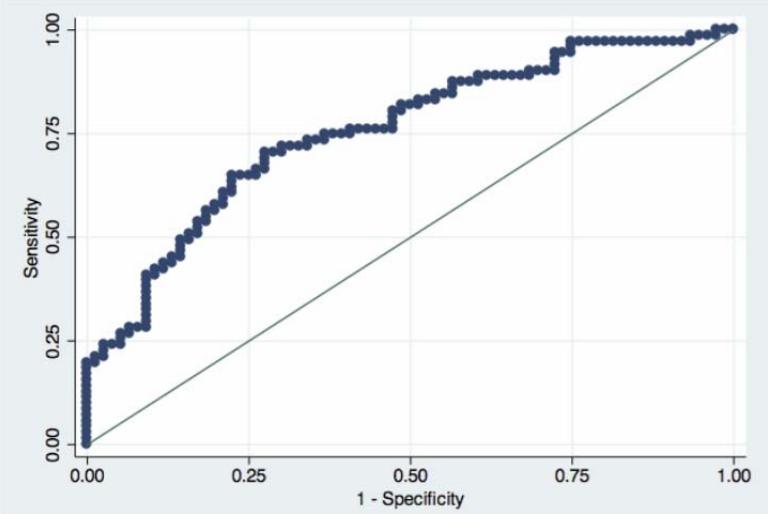
Now recently work had been done on using exosome RNA extracts to assess diagnostic information on PCa. We examine some of the data recently presented by McKiernan et al regarding the testing approach employed by Exosomedx. The results show significant improvement of the area under the ROC from standard PSA tests.

From McKiernan et al:

ROC curve for exoRNA PCA3 expression (n=143, PNB negative=73, PNB positive=70; primary biopsy=71.5%, history of negative biopsy=28.5%) ROC = 0.68



4-gene signature (n=147; PNB negative n=76, PNB positive n=71; primary biopsy=82.4%, history of negative biopsy=17.6%) ROC = 0.75



We demonstrate that urinary exoRNA, without the need for prostatic massage, can be used to non-invasively examine prostatic gene expression This information can accurately predict the likelihood of having a positive vs. negative biopsy result Additionally, this information can also help differentiate low vs. high grade cancers at time of surgery The unique properties of urinary exosomes simplifies sample handing, obviates sample variability, and minimizes patient discomfort

Other addition work in this area has been done by Lahmann. Lahmann et al state:

…we have extended the recent discovery by Valadi and colleagues that exosomes contain small RNA. In this important study, the authors showed that exosomes released from mouse and human mast cells contain an abundance of small mRNAs and microRNAs.

In addition, it was established that RNA from mouse mast cells was transferable to recipient human mast cells and can be translated after entering the recipient cell. In our preliminary analysis, we have found that prematurely senescent (irradiation-induced) 22Rv1 cells also release exosomes containing substantial amounts of small RNAs (Fig. 6D; <100 bp).

To confirm that the nucleotides detected by agarose gel electrophoresis were RNA, exosome extracts were treated with either RNase or DNase in solution.

4.3 SURVIVIN; ANOTHER TARGET?

Survivin is also a target of some interest in prostate cancer. It allows for the continual proliferation of cancer cells and inhibits apoptosis.

As Kahn et al state:

Survivin is expressed in prostate cancer (PCa), and its downregulation sensitizes PCa cells to chemotherapeutic agents in vitro and in vivo. Small membrane-bound vesicles called exosomes, secreted from the endosomal membrane compartment, contain RNA and protein that they readily transport via exosome internalization into recipient cells. Recent studies demonstrate that Survivin exists in plasma exosomes from both normal, BPH and PCa subjects.

The relative amounts of exosomal Survivin in PCa plasma was significantly higher than in those with pre-inflammatory BPH and control plasma. This differential expression of exosomal Survivin was seen with both newly diagnosed and advanced PCa subjects with high or low-grade cancers. Analysis of plasma exosomal Survivin levels may offer a convenient tool for diagnosing or monitoring PCa and may, as it is elevated in low as well as high Gleason scored samples, be used for early detection. progress has shown that tumor-derived exosomes play multiple roles in tumor growth and metastasis and may produce these functions via immune escape, tumor invasion and angiogenesis.

Furthermore, exosome analysis may provide novel biomarkers to diagnose or monitor PCa treatment.

They continue:

The processes of both cell survival and cell death have involved highly regulated signaling pathways that are currently the subject of intense investigation. It is known that regulation of apoptosis has a central role in the development of prostate cancer and its progression to an androgen-independent state, which is due, in part to up regulation of antiapoptotic genes after androgen deprivation. Several lines of evidence suggest that one of the main events associated with progression after therapeutic failure is increased resistance to apoptosis, mainly due to the up regulation of antiapoptotic genes, including Bcl-2, Bcl-XL, Mcl-1, and Survivin.

Survivin, an inhibitor-of-apoptosis (IAP) protein family member, is associated with PCa development, progression, and drug resistance. Recent evidence indicates that the overexpression of Survivin in PCa tumors is associated with poor prognosis and increased tumor recurrence. In contrast, it has also been shown that knockdown of survivin expression by siRNAs enhances the chemosensitivity of prostate cancer cells, reducing tumorigenicity

From Lin et al we have:

Given the numerous new agents for the treatment of patients with castration resistant prostate cancer (CRPC), identification of biological markers is essential to predicting response and providing a rationale for sequencing treatments. Microvesicles, including exosomes, are small lipid bilayer vesicles released from all cells into bodily fluids. Exosomes, which carry high integrity RNA from their parent cells, can be used to reliably interrogate the transcriptional profile of various organs in a non-invasive manner. Using urinary exosomes we examined Survivin expression, an inhibitor of apoptosis implicated in hormone independent tumor growth, in different disease states of men with advanced prostate cancer….

Using urinary exosomal RNA, Survivin levels are elevated in men with CRPC. This non-invasive assay may be utilized to follow prostate cancer patients over time and monitor gene expression during treatment, giving greater insight into the molecular changes that occur during disease progression and response to treatment.

5 OBSERVATIONS

Exosomes represent an interesting and potentially valuable tool in the diagnosis and prognosis of certain cancers. There are issues however that we must consider in their use.

5.1 CONSIDERATIONS

As with many tests of this type there are several critical considerations. We now mention a few:

Source Site of the Exosome: The test determines diagnostic or prognostic values for sample taken from a non-invasive site such as the blood or urine. The problem is that we do not necessarily know from where the exosome may have come from. Let us examine PCa as an example. The exosome in the urine may most likely have arisen from the urinary tract, namely somewhere from the nephron, through the bladder then near the prostate. Thus one may ask just where these come from did.

Source Cell of the Exosome: The second question is; from what cell did this arise? This may beg the question of the stem cell issue. As we have discussed before, the PCa stem cell may be the dominant cell and exosome may be from other daughter cells not being a stem cell.

Timing: How frequently should these samples be made and over what period of time? This is key question say when doing a urine voiding sample.

5.2 CIRCULATING CANCER CELLS

The challenge is determining of a cancer has metastasized is to find out where and how much. The classic approach is to look at the local draining lymph nodes and see if has gone there. However the cancer cells may often escape through the blood system and not the lymph system. Consider ocular melanoma, there is no lymph system connection and it spreads by hematological means only.

That means that by examining the blood we should be able to find the wandering malignant cells, at least in theory. In a recent release by MedGadget the article relates developments at MGH in Boston as follows:

Circulating tumor cells (CTCs) are shed by primary tumors and allow the cancer to metastasize to the distant sites. While this is a devastating tool in cancer's war chest, it offers clinicians a marker through which to diagnose and monitor progress of the disease. Since the discovery of CTCs over a hundred years ago, researchers have been developing ever more sensitive methods of capturing them since they're extremely rare in whole blood.

In a recent development by Ozkumur et al at MGH the authors state:

Circulating tumor cells (CTCs) are shed into the bloodstream from primary and metastatic tumor deposits. Their isolation and analysis hold great promise for the early detection of invasive

cancer and the management of advanced disease, but technological hurdles have limited their broad clinical utility. We describe an inertial focusing–enhanced microfluidic CTC capture platform, termed "CTC-iChip," that is capable of sorting rare CTCs from whole blood at 107 cells/s.

Most importantly, the iChip is capable of isolating CTCs using strategies that are either dependent or independent of tumor membrane epitopes, and thus applicable to virtually all cancers. We specifically demonstrate the use of the iChip in an expanded set of both epithelial and nonepithelial cancers including lung, prostate, pancreas, breast, and melanoma.

The sorting of CTCs as unfixed cells in solution allows for the application of high-quality clinically standardized morphological and immunohistochemical analyses, as well as RNA-based single-cell molecular characterization. The combination of an unbiased, broadly applicable, high-throughput, and automatable rare cell sorting technology with generally accepted molecular assays and cytology standards will enable the integration of CTC-based diagnostics into the clinical management of cancer.

There are several problems here however:

1. As we had demonstrated in some of our prior analysis, blood borne cancer cells are rare, but more importantly they are cells which are coming from and going to organs. Namely they are in transit, from whence and to where we do not know.

2. The genetic states of each of these wandering cells may be a marker of from whence it came. The problem is that we do not fully understand this genetic mutation process, and in fact as we have shown before it may actually be a Markov like chain process.

3. Understanding this change in cells may be of significant therapeutic value. However this again is uncertain given our current state of knowledge.

4. Again we come back to the cancer stem cell and ask if the few cells we find in the blood stream are the right cells to examine.

However this advance could provide significant data to allow us to expand the understanding of mutating cancer cells.

5.3 MORE ON CIRCULATING CELLS

There was a Press Release today covered in Technology Review regarding the chip which will catch cancer cells in the blood. The Tech Review states:

The prototype, developed by Mehmet Toner and collaborators at MGH, consists of a business-card-size silicon chip dotted with tens of thousands of microscopic posts. Each post is coated with a molecule that binds to a protein unique to cells from a specific type of tumor, such as breast, lung, or prostate cancer. As blood flows through the chip, tumor cells stick to the posts. In 2007, the researchers first showed that the chip could capture these rare cells--which make up

just one in a billion cells in blood--in high enough numbers to analyze them for molecular markers.

The ultimate goal is to use the device to tailor cancer treatments to individual patients by monitoring cancer cell counts and by identifying the molecular attributes of an individual's cancer. For example, specific markers can highlight a more aggressive form of cancer or a tumor that will respond to specific cancer drugs, while genetic changes in the tumor might signal the need to change treatments. MGH and four other research institutions, have already received a $15 million grant from the organization Stand Up to Cancer to test the prototype. But that technology is expensive and complicated to use, with each chip costing about $500, according to the Boston Globe.

There are however some concerns:

1. By the time the cancer cells are freely circulating they may most likely have metastasized. Take a prostate cancer, PCa, which may be Gleason 5 to 7, and it is most likely still encapsulated. It must generally get to an 8-10 to metastasize. At that point there are PCa cells circulating. If so, what is the benefit, because the PSA most likely is well over 10.

2. There is the conundrum of the stem cell theory of cancer, namely that there are stem cells and these are the cells which will spread the cancer. Can we distinguish stem cells from plain old cancer cells. Not yet clear we can do it if we have them, no less looking in the blood stream.

3. A cell which may be cancerous, say morphologically with large nucleoli and other markers must be examined in more detail. Say PCa, can we see if it has no PTEN, what of mTOR, c-myc, and the list of other genes, and what of the miRNAs which we may now suspect to be markers as well. We have to look at a single cell. May be tough, since it is already tough in a path study.

Not that this is not a good idea, but the Press seems to have said more than is there. Shame, since if it does not deliver to the level expected that in itself is negative. Nano technology has greater potential possibly, since with it we can send them through organs with surface collectors and look for the cell markers.

6 REFERENCES

1. Camussi, G., et al, Exosome/microvesicle-mediated epigenetic reprogramming of cells , Am J Cancer Res 2011;1(1):98-110
2. Duijvesz, D., et al, Exosomes as Biomarker Treasure Chests for Prostate Cancer, European Urology, V 59, 2011, pp 823-831.
3. Halberg, N., et al, microRNA regulation of cancer–endothelial interactions: vesicular microRNAs on the move, The EMBO Journal (2012) 31, 3509–3510.
4. Hood, J., et al, Exosomes released by melanoma cells prepare sentinel lymph nodes for tumor metastasis, Cancer Res Published OnlineFirst April 8, 2011.
5. Kahn, S., et al, Plasma-Derived Exosomal Survivin, a Plausible Biomarker for Early Detection of Prostate Cancer, PLOS ONE, October 2012, Volume 7, Issue 10.
6. Keller, S., et al, Exosomes: From biogenesis and secretion to biological function, Immunology Letters 107 (2006) 102–108.
7. Lehmann, B., et al, Senescence-Associated Exosome Release from Human Prostate Cancer Cells, Cancer Res 2008;68:7864-7871.
8. Lin, J., et al, The Novel Use of Urinary Exosomes to Examine Survivin Expression in Castration Resistant Prostate Cancer, Annual Meetings of the American Urological Association, Atlanta GA (May 2012)
9. McKiernan, J., et al, Urinary exosomes/microvesicles as a non-invasive platform for prostate cancer analysis, AUA — American Urological Association — San Diego, CA (May 2013). http://www.exosomedx.com/science-exosomes/abstracts/AUA_Exosomes_RP-Urinary-exosomes-noninvasive-platform-for-prostate-cancer-analysis.pdf
10. Ozkumur, E., Inertial Focusing for Tumor Antigen–Dependent and –Independent Sorting of Rare Circulating Tumor Cells, Sci Transl Med 3 April 2013: Vol. 5, Issue 179, p. 179
11. Peinado, H., et al, Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET, nature medicine VOLUME 18, NUMBER 6, JUNE 2012.
12. Rana, S., et al, Exosomal Tumor MicroRNA Modulates Premetastatic Organ Cells, Neoplasia, Volume 15 Number 3 March 2013 pp. 281–295.
13. Skog, J., et al, Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers, Nature Cell Biology 10, 1470 - 1476 (2008).
14. Taylor, D., C. Taylor, MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer, Gynecologic Oncology 110 (2008) 13–21.
15. Xaio, D, et al, Identifying mRNA, MicroRNA and Protein Profiles of Melanoma Exosomes, PLOS ONE, www.plosone.org , October 2012,Volume 7 Issue 10