Cancer Biology & Therapy 12:3, 159-164; August 1, 2011; © 2011 Landes Bioscience

Vesicle transfer and cell fusion

Emerging concepts of cell-cell communication in the tumor microenvironment

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Keywords: exosome, cell-fusion, cancer, vesicle, tumor microenvironment

Abbreviations: HERV, human endogenous retrovirus; HPV, human papillomavirus; EBV, Epstein–Barr virus; CMV, cytomegalovirus; MGC, multinucleate giant cell; IL-4, Interleukin-4; IL-13, Interleukin-13; GM-CSF, granulocyte-macrophage colony-stimulating factor; M-CSF, macrophage colony-stimulating factor; RANK, receptor activator of nuclear factor-κB; RANKL, receptor activator of nuclear factor-κB ligand; SIRPα, signal regulatory protein alpha; MM, multiple myeloma; DCs, dendritic cells; Baff, B-cell activating factor; APRIL, A proliferation-inducing ligand; TSP-1, Thombospondin-1; RNAi, RNA interference; BMDCs, bone marrow-derived cells; YFP, yellow fluorescent protein; MV, microvesicle; ILV, intraluminal vesicle; MVB, multivesicular body; GBM, glioblastoma multiforme; ARF6, ADP-ribosylation factor 6; EMT, epithelial-mesenchymal transition; CLL, chronic lymphocytic leukemia; RTK, receptor tyrosine kinase; VEGF, vascular endothelial growth factor; DRF3/Dia2, diaphanous related formin 3; EGF, epidermal growth factor; PLAP, placental alkaline phosphatase; PLA2, phospholipase A2; PLD2, phospholipase D2; 15d-PGJ2, 15-Deoxy-Delta-12,14-prostaglandin J2; PGE2, Prostaglandin E2; TDEs, tumor-derived exosomes; CTLs, cytotoxic T lymphocytes; Tregs, regulatory T cells; TGFβ1, transforming growth factor beta 1; IL-10, Interleukin-10; Ab, antibody; MDSCs, myeloid derived suppressor cells; FasL, Fas ligand; NK, Natural Killer; MyD88, myeloid differentiation primary response gene (88); MHC, major histocompatibility complex

Cell-cell fusion and vesicle-mediated transfer are fundamental biological processes that are emerging as novel mechanisms for re-programming cells in the tumor microenvironment. Both cell-cell fusion and intercellular transfer of vesicles (including microvesicles and exosomes) allow for the transfer of information among tumor cells, between tumor cells and tumor stroma, and between tumor cells and the host immune system, which could have profound implications for our understanding of tumor initiation and progression. The National Cancer Institute's Division of Cancer Biology sponsored a recent workshop (December 15–17, 2010) entitled, *Vesicle Transfer and Cell Fusion: Emerging Concepts of Cell-Cell Communication in the Tumor Microenvironment* to assess the current state of the science in these two scientific areas. Co-chaired by Drs. Huang-Ge Zhang (University of Louisville) and Madhav Dhodapkar (Yale University) this workshop brought together, for the first time at the NIH, leaders in the field to assess the effects of vesicle transfer and cell-cell fusion on cancer initiation, progression and metastasis. This meeting report includes brief summaries of the presentations and identifies the major questions, roadblocks, and opportunities. The meeting report is presented here to highlight research priorities and to stimulate basic and translational research efforts to better understand the contributions of cell-cell fusion and vesicle transfer to cancer.

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Submitted: 06/24/11; Accepted: 06/24/11
DOI: 10.4161/cbt.12.3.17032

Introduction

Mutations and epigenetic events are thought to be the principal pathways by which transformed cells acquire the abilities to escape cell cycle control, resist chemotherapy, and metastasize. However, recent evidence also points to novel mechanisms, including cell-cell fusion and vesicle-mediated transfer that may provide alternative pathways by which tumor cells can acquire these capabilities and sculpt the tumor microenvironment. While cell-cell fusion and vesicle-mediated transfer are separate mechanisms both involve membrane fusion events which may allow transfer of information among tumor cells, between tumor cells and tumor stroma, and between tumor cells and the host immune system which could have profound effects on cancer progression. A recent workshop organized by the Division of Cancer Biology (DCB) at the National Cancer Institute (NCI), and chaired by Madhav Dhodapkar and Huang-Ge Zhang, assembled experts in cell fusion and vesicular biology to summarize the current state of the knowledge and assess the potential role(s) of cell-cell fusion and vesicle transfer in cancer progression. The meeting summary is presented here to highlight research priorities and stimulate basic and translational research efforts to better understand the contributions of cell-cell fusion and vesicle biogenesis and transfer to cancer.

Cell Fusion in Carcinogenesis

Yuri Lazebnik (Cold Spring Harbor Laboratory) introduced cell fusion as an essential process in developmental biology involved

in fertilization, syncytiotrophoblast formation, and muscle and bone development.[1] The biological mechanisms that underlie these fundamental processes are not clearly understood, raising the possibility that deregulating these mechanisms could contribute to disease and cancer. Human trophoblasts, for example express fusogenic proteins encoded by an endogenous retrovirus (HERV) that mediate syncytia formation. HERVs comprise approximately 8% of the human genome and many cancers express HERV encoded fusogenic proteins suggesting the potential that HERVs contribute to cancer by generating tumor cells permissive for cell fusion. In addition, a number of common endemic viruses such as HPV, EBV, and CMV encode fusogenic proteins that can generate epithelial multinucleate cells by different mechanisms. Experimental observations suggest that endogenous retrovirus- or infectious virus-mediated cell fusions could potentially contribute to cancer etiology in at least two ways: either by destabilizing genomes or epigenomes or by providing a tumor cell with new capabilities to invade surrounding tissues and remote sites.

Following on this theme, Agnes Vignery (Yale University) provided an overview of the unique ability of macrophage to fuse, generating either multinucleate giant cells (MGCs) in response to pathogens or foreign bodies or osteoclasts involved in bone remodeling. In in vitro culture, macrophages readily fuse in response to cytokines; IL-4/IL-13 and GM-CSF leads to MGC formation whereas M-CSF and RANKL generate osteoclasts. It is important to note that macrophage fusion enables novel behaviors like bone resorption. In addition, self-nonself recognition molecules CD47, its ligand SIRPα, and CD200 were highly induced at the outset of cell fusion.[2] These receptors play a major role in regulating macrophage phagocytosis and lead to the intriguing suggestion that the machinery of self-nonself recognition also regulates macrophage-macrophage cell fusion. Thus, macrophage-tumor cell fusion could endow tumor cells with properties of macrophages such as the ability to invade and seed distant body sites which are characteristics of aggressive metastatic cancers.

Madhav Dhodapkar (Yale University) expanded the link between self-nonself discrimination and cell fusion in the setting of multiple myeloma (MM). Characteristics of MM include chronic inflammation associated with osteolytic lesions and a marked enrichment of dendritic cells (DCs). A central question in MM is how DCs and MM cells affect each other in the tumor microenvironment. DCs alter MM differentiation and survival through RANK/RANKL and Baff/APRIL cytokines, in part by upregulating the anti-apoptotic molecule BCL-6. In contrast, MM cells stimulate DC fusion to form osteoclasts which contribute to lytic lesions. Expression analysis revealed that upregulation of CD47 (on MM cells) and TSP-1 (on DCs) was necessary for fusion. Blocking the CD47-TSP-1 interaction by RNAi or with a specific anti-TSP-1 antibody inhibited osteoclast generation, both in vitro and in vivo.[3] Taken together, these findings suggest that expression of CD47 on tumor cells delivers a fusogenic signal via TSP-1 inducing DC fusion. As discussed above, another ligand for CD47 is SIRPα which is the best known of the innate "do not eat me" signals that prevent phagocytosis. Thus, the same ligands that deliver a "do not eat me" signal in one context (phagocytosis) might well instruct DCs to fuse in the MM microenvironment.

Melissa Wong (Oregon Health and Science University) presented elegant model systems demonstrating in vivo cell fusion between bone marrow-derived cells (BMDCs) and differentiated intestinal epithelia in response to injury. Whereas various BMDC lineages supported a low-level of fusion in the intestinal epithelium, macrophages generated the most robust levels. In the setting of cancer, cell fusion was demonstrated by transferring cre-recombinase expressing MCA38 colorectal cancer cells into recipients expressing a YFP cre-reporter. YFP expressing tumor cells reflected fusion between the tumor cell and host BMDCs. Expression analysis of macrophage-MCA38 cell fusion hybrids revealed expression of a unique suite of genes not expressed by parental cell lines and acquisition of macrophage characteristics.[4] These data reinforce the idea that metastatic cancer cells share many functional properties with macrophages including the ability to induce angiogenesis, remodel the extracellular matrix, and the ability to intravasate and move throughout the body and extravasate at distant sites.

John Pawelek (Yale University) described ongoing efforts to generate a direct demonstration that cell fusion plays a role in human cancer. Patients that had previously received allogeneic bone marrow transplants and subsequently developed secondary tumors could provide the genetic evidence necessary to validate macrophage-tumor cell fusion in human cancer. In one case, a male that had received a bone marrow transplant 10 y earlier for lymphoma later developed melanoma with metastasis to lymph node and brain. Tumor fragments containing only melanoma cells, free of BMDCs, were isolated by staining for common leukocyte antigen markers and microdissection. DNA from tumor cells contained a mixture of donor and host alleles at some loci suggesting cell fusion between host tumor cells and donor BMDCs. While this study needs to be expanded to more cases, this approach could provide genetic evidence of hybridization in vivo and verify a role for cell fusion in human tumorigenesis.

Leonid Chernomordik (NICHD, NIH) discussed the dynamics of membrane fusion events in cell-cell fusion and other fusion processes including exocytosis and protein trafficking. Although distinct cellular machineries mediate membrane fusion events temporally and compartmentally, there are common motifs to these processes. For example, fusion between two membrane bilayers is regulated by the lipid composition of the membranes and the presence of protein fusogens. Whereas viral fusogens are efficient and well-studied, less is known about physiological cellular fusogens or their activities. Fusion of myoblasts into myotubes is associated with externalization of phosphatidylserine to the outer membrane leaflet and an increased presence of phosphatidylserine-binding annexin proteins. Interestingly, tumor cells have been reported to be annexin A5 positive and this may represent one pathway that facilitates cell-cell fusion in the tumor microenvironment.

Xin Lu (Dana-Farber Cancer Institute) asked whether cell fusion regulates the organtotropism of metastases. For example, lung, liver, bone and brain are frequent sites of metastasis in breast cancer. In contrast, prostate cancer generally metastasizes

to bone. To answer this question two MDA-MD-231 human breast cancer cell sublines, one metastatic to lung and the other to bone, were differentially labeled and spontaneous fusion hybrids were examined for the ability to metastasize to specific organ sites. Whereas control self-fusions retained the original organotropism of the parental lines, hybrid-fusions could metastasize to both lung and bone. Thus, hybrids acquire metastatic competencies of both parental lines. In addition, these investigators found that spontaneous ploidy duplication, a direct consequence of cell fusion, could enhance metastatic ability in specific organs where tumor cell size increase caused more seeding in the vasculature (e.g., in lung and brain, but not bone).[5]

The observation that cell fusion, and the multinucleate cells that result, are common in developmental biology suggest that cells have an inherent ability to tolerate polyploidy. Multinucleated cells are commonly observed in cancer but this is generally assumed to be the result of failed mitosis or cytokinesis rather than cell fusion. However, since cancer cells are well known to co-opt existing biological processes to further their survival, cell fusion may represent one such process. Cell fusion can in principle, lead to all the characteristics common to cancer, including genomic instability, aneuploidy, cancer initiating stem-like properties, and acquisition of new behavioral traits like multi-drug resistance or metastatic ability. Lastly, cell fusion could also affect the organotropism of metastases. A recurrent theme in the workshop presentations was that cell fusion enabled novel cellular behaviors in the hybrid cell that were not present in the fusion partners. However, whether cell fusion generates hybrid cells with oncogenic potential remains a tantalizing yet unresolved question.

Vesicle Transfer in Carcinogenesis

Tumor cells release different types of vesicles including shed microvesicles (MVs), secreted exosomes, microparticles containing retroviral elements, membrane blebs and apoptotic bodies. Each vesicle subclass possesses distinct size characteristics, lipid compositions, membrane-associated proteins, and cargos. Graça Raposo (Institut Curie) opened the workshop with an overview of vesicle biology. During endosome maturation intraluminal vesicles (ILVs) of multivesicular bodies (MVBs) are formed by invagination of the endosomal limiting membrane. MVBs generally fuse with lysosomes to degrade their contents, but can also fuse with the plasma membrane to secrete the ILVs into the extracellular environment. These ILVs are then called exosomes. Although exosomes share some characteristics with other vesicle subclasses they can be distinguished by size, protein signature, and endosomal biogenesis. Exosomes range in size from 50–80 nm and differ from shed MVs which are derived from the plasma membrane, are larger (> 100 nm), and have a distinct protein signature. Membrane blebs and apoptotic bodies are in the size range of 1–2 microns. Pioneering studies in the mid 1990s demonstrated that dendritic cell-derived exosomes loaded with tumor peptide-MHC complexes could stimulate antitumor immune responses. Exosomes are now considered as potential vehicles for a variety of bioactive molecules that play critical roles in regulating immune responses, regulating inflammation, and mediating crosstalk between tumor cells and the tumor stroma.

Xandra Breakefield (Massachusetts General Hospital) discussed the role of tumor-derived MVs and associated cargo as serum biomarkers of cancer genetics, progression and recurrence. Healthy individuals have about 10^{11} MVs per ml serum while cancer patients have approximately 10-fold more and these tumor-derived MVs have cargos representing cancer-specific signatures. For example, MVs generated by glioblastoma multiforme (GBM) cells contain more RNA and also DNA (which is not generally seen in MVs) compared with MVs released by normal cells. Thus, specific mutant mRNAs, differentially expressed nucleic acids and proteins in tumor MVs may serve as sensitive and highly specific indicators of tumor genetic status and progression.[6] However, tumor-derived MVs represent only a subpopulation within a pool of normal MVs derived from platelets, endothelial cells and neutrophils. Distinguishing among the various vesicle subtypes and selecting for tumor microvesicles will be a significant challenge for future studies.

Michael Paulaitis (Ohio State University) presented an elegant scheme to separate vesicles using a two-step technique separating first by size, then by specific capture on a microarray based on surface receptor status, and finally performing nano-droplet quantitative RT-PCR analysis to analyze vesicle contents. The approach to isolate vesicles on the basis of size and surface markers could differentiate among the many types of vesicles and allow specific quantification of their respective cargos.

Crislyn D'Souza-Schorey (University of Notre Dame) discussed the role of the Ras-like protein ARF6 in cancer progression. Activated ARF6, which is linked to regulating endocytic trafficking and peripheral actin remodeling, is markedly upregulated at the invasive fronts of tumors. Stimuli that promote tumor invasion also upregulate ARF6 and invasiveness can be blocked by expression of dominant negative ARF6. Impaired ARF6 in invasive tumor cell lines, inhibits an early endosomal recycling pathway that appears to direct some cargo to shedding MVs. However, the specific ARF6 pathway(s) that regulate MV shedding need to be identified. These MVs express MHC class I, ARF6, and integrin receptors but not transferrin or proteins enriched in invadopodia, suggesting that MV cargo is selectively recruited.[7] In addition, MVs are enriched in proteases which likely contribute to degradation of matrices at areas of active tumor invasion. Determining how distinct cargos are specifically recruited to microvesicles and contribute to cancer progression will be important goals for ongoing studies.

Similarly, Matthew Ringel (Ohio State University) compared gene expression signatures from central and invasive fronts in thyroid cancer. Consistent with epithelial-mesenchymal transition (EMT) at the invasive front, expression of signaling and cell adhesion molecules was reduced compared with the central part of the tumor. Vimentin, osteopontin and RUNX2, factors involved in EMT transition were increased at the invasive fronts. Interestingly, distinct RAB proteins associated with MVB biogenesis were also increased in the central regions in comparison to the invasive fronts. Future studies will examine whether tumor-derived MVs represent a horizontal transfer of information

among tumor cells and between tumor cells and the stroma that modulate EMT, invasion, and matrix degradation. Thus, specific MV associated cargo could represent potential biomarkers for cancer invasion.

The role of MV-stromal cell crosstalk in the setting of chronic lymphocytic leukemia was addressed by Neil Kay (Mayo Clinic, Rochester, MA). Shed MVs from control and CLL patients were isolated from patient sera and characterized. MVs from CLL patients were increased relative to controls, heterogeneous in size but consistent with a shed MV origin, and Annexin positive. Analysis by mass spectroscopy revealed over 700 proteins including transcription factors, signal transducers, adhesion molecules, cell surface receptors, and anti-apoptotic proteins. Incubation of MVs from CLL cells with primary marrow stromal cells led to elevated AKT and receptor tyrosine kinase (RTK) expression and/or activation which appeared to be associated with more aggressive CLL disease. In addition, the Akt signal pathways and VEGF secretion levels of CLL marrow stromal cells appear very different compared with stromal cells from healthy controls.[8] Taken together, these results indicate that CLL shed MVs induce aberrant stromal function and modulate the tumor microenvironment. Thus, MVs and exosomes may play significant roles in conditioning the tumor microenvironment to favor tumor development and progression.

Along the spectrum of MV subclasses are larger membrane blebs that are being pursued by Michael Freeman (Harvard Medical School). Knockdown of diaphanous related formin 3 (DRF3/Dia2), an actin nucleating protein family member, by RNAi reduced aggregation, enhanced amoeboid-type cell motility, and membrane blebbing in DU145 and LNCaP human prostate cancer cell lines. Membrane blebs were further increased in response to epidermal growth factor (EGF) stimulation suggesting these MVs were not associated with cell death. DRF3 is lost at high frequency in aggressive metastatic prostate cancers, compared with patients with local disease, suggesting DRF3 regulates acquisition of a metastatic phenotype. Characterization of the MVs by semi quantitative proteomics revealed the presence of multiple signaling proteins, oncoproteins, and the integral membrane protein caveolin 1 which is a plasma biomarker of metastatic prostate cancer.

Dorothy Lewis (UT Health) demonstrated that circulating apoptotic bodies produced as a result of trophoblast cell death can be specifically identified in maternal plasma by the presence of fetal markers HLA-G and placental alkaline phosphatase (PLAP), which are not present in serum from non-pregnant women. Apoptotic bodies also contain fetal DNA which can be used to screen for genetic markers of disease. Necrotic trophoblast cell death leads to quantitatively fewer and qualitatively distinct vesicles. In particular, apoptotic bodies contain nicked DNA and more lipids. Vesicle content, beyond serving as a biomarker of disease could also reveal cell of origin, biogenesis, and potential target cells.

The impact of phospholipid composition on exosome function was discussed by Michel Record (INSERM). Exosomes are rapidly internalized after contact with recipient cells and sorted to the MVB suggesting that donor and recipient vesicular information could be mixed, resorted, or concentrated for delivery to downstream recipient cells. Exosome membranes contain cholesterol, sphingomyelin, and bioactive lipids but do not display lipid asymmetry. Proteomic analyses revealed that exosomes contain distinct protein signatures, in particular phospholipases (PLD2, PLA2) and cyclooxygenases. PLA2 generates arachidonic acid, further processed into bioactive leukotrienes. Cyclooxygenases convert arachidonic acid to prostaglandins PGE2 (which is immunosuppressive and supports tumor progression) and 15d-PGJ2 (which suppresses tumor growth). Both PGE2 and 15d-PGJ2 are strikingly enriched in exosomes compared with parental cells.[9] Taken together, these findings suggest that exosomes could regulate the balance between tumor growth and immunosuppression during cancer progression.

Following this theme Theresa Whiteside (University of Pittsburgh) demonstrated the ability of tumor-derived exosomes (TDEs) to suppress antitumor cytotoxic T lymphocytes (CTLs) and promote suppressive regulatory T cells (Tregs). TDEs from cancer patient sera were shown to express FasL and induce caspase-3-dependent T-lymphocyte apoptosis. Neutralizing anti-Fas antibody partially blocked apoptosis induction. In other studies, TDEs also increased the frequency and function of $CD4^+CD25^{hi}$ $FoxP3^+$ Treg cells. TDE carry TGFβ1 and IL-10 and addition of neutralizing Abs to TGFβ1 and IL-10 diminished the ability of TDEs to expand Treg numbers and function.[10] Further, leukemic blast-derived exosomes isolated from the sera of patients with acute myeloid leukemia were shown to suppress NK cell activity via membrane-associated TGFβ1. The ability of TDEs to suppress antitumor immune responses should be considered a significant tumor escape mechanism

Huang-Ge Zhang (Louisville University) highlighted additional mechanisms by which TDEs suppress antitumor effector cells in the tumor microenvironment.[11] TDEs impair antitumor immunity by blocking the differentiation of immature DC into antigen presenting cells, inducing the accumulation of $GR1^+CD11b^+$ myeloid derived suppressor cells (MDSCs) and promoting metastasis. Generation of MDSCs and metastasis were reduced in MyD88-deficient mice suggesting a role for Toll-like receptors. While TDEs possess the ability to suppress antitumor immunity, they are also an excellent source of tumor antigens and MHC molecules. Thus, the challenge is to reverse TDE induced immune suppression while preserving the ability of TDEs to stimulate antitumor immunity. A promising approach for future studies would be to use exosomes to specifically destroy tumor cells by manipulating exosome content and targeting specificity.

Tumor cells shed or exocytose a variety of vesicles that express transmembrane proteins and a complex cargo of bioactive nucleic acids (DNA, mRNA and microRNA), proteins, oncoproteins, and lipids. Tumor-derived MVs and exosomes have been reported to repress antitumor immune responses, to induce stromal cells to support tumor growth, and assist tumor escape demonstrating profound effects both locally in the tumor microenvironment and systemically. Taken together, these studies are demonstrating that tumor-derived MVs can condition the tumor microenvironment to promote cancer progression and metastasis and also serve as biomarkers of disease progression. Investigations in this area should be encouraged.

At the conclusion of the workshop, a general discussion of the topics presented identified outstanding questions which led to the following research priorities:

Microvesicles/exosomes:

•A standardized nomenclature is needed to identify and distinguish between the various vesicle subclasses.

•Defining parameters such as size, cargo (including DNA, RNA, microRNA, protein and lipid composition), surface ligands, and functional downstream effects for the various vesicle subclasses need to be accurately defined.

•Methods to study the impact of vesicle impact on local and systemic target cells or tissues need to be identified in order that responses can be accurately detected and quantitated.

•Conditions that modulate vesicle formation and release (i.e., hypoxia or inflammation) and what features of the producer cells (i.e., adherent vs. non-adherent cells) affect vesicle biogenesis, content, and release need to be defined.

•Specific markers or signatures that can be used as diagnostics or incorporated into protocols for vesicle capture and/or characterization are needed. Markers of vesicle trafficking inside the cell would be useful.

•Protocols for measuring, separating, and purifying vesicles from body fluids or tissues need to be standardized.

•A better understanding of the general biological mechanisms or pathways by which vesicles are generated, how vesicle cargo is recruited, and how vesicles are released is required.

•There needs to be a better understanding of how vesicle cargo is recruited and whether cargo composition is regulated constitutively or by activating factors.

•Agents that specifically regulate or restrict MV release need to be identified in to order to determine the biological effects caused by cessation of vesicle release.

•How vesicles transmit signals and impact target cell behavior needs to be better understood. What role(s) are played by receptor-ligand signaling events vs. transfer of vesicle cargo need to be defined?

•What determines whether tumor-derived MVs are immunogenic or immunosuppressive?

• Is vesicle formation and release necessary for carcinogenesis? Can they serve as biomarkers for cancer status?

• Can MVs be engineered to encapsulate specific luminal products and express targeting molecules and used therapeutically to treat disease.

Fusion:

•There is a need to better understand the frequency of cell fusion events in vivo and their clinical and biological significance in human cancer.

•Do cell fusion events contribute to genomic instability in cancer?

•Can cell fusion events lead to genetic modification of the tumor microenvironment?

•Can cell fusion result in the acquisition of therapeutic resistance?

•Can cell fusion result in reprogramming target cells with new properties that augment cancer progression and/or metastasis?

•Can bone marrow derived cell fusion with transformed cells generate cancer stem cells?

•There is a need to understand the mechanistic means of cell fusion and how the process can be regulated.

•The role of oncogenic viruses and endogenous retroviruses in mediating cell fusion event needs further investigation.

•Do hybrid cells have any hallmark features that distinguish them and can these be exploited to demonstrate the role on cell fusion in cancer progression?

•Do exosomes play a role in mediating cell fusion?

•How do organelles interact in fused cells?

•How heterogeneous are the progeny of cell fusion and how is this heterogeneity determined?

•How does cell fusion affect epigenetic regulation of the parental cells? Does cell fusion cause emergent properties (absent in the parents), including properties relevant to cancer?

•Do known oncogenic events facilitate or inhibit cell fusion?

•What is the biological basis for stem cell fusion?

Attendees

Xandra Breakefield (Massachusetts General Hospital, Boston, MA), Leonid Chernomordik (NICHD/NIH, Bethesda, MD), Crislyn D'Souza-Schorey (University of Notre Dame, South Bend, IN), Madhav Dhodapkar (Yale University, New Haven, CT), Michael Freeman (Harvard Medical School, Boston, MA), Dan Gallahan, (NCI, Bethesda, MD), Kevin Howcroft (NCI, Bethesda, MD), Neil Kay (Mayo Clinic, Rochester, MN), Peter Kurre (Oregon Health and Science University, Portland, OR), Yuri Lazebnik (Cold Spring Harbor Laboratory, Laurel Hollow, NY), Dorothy Lewis (UT Health, Houston, TX), Xin Lu (Dana-Farber Cancer Institute, Boston, MA), Susan McCarthy, (NCI, Bethesda, MD), Suresh Mohla (NCI, Bethesda, MD), Allan Mufson, (NCI, Bethesda, MD), Michael Paulaitis (Ohio State University, Columbus, OH), John Pawelek (Yale University, New Haven, CT), Graça Raposo (Institut Curie, Paris, France), Michel Record (INSERM 1037, CRCT, Toulouse, France), Matthew Ringel (Ohio State University, Columbus, OH), Dinah Singer (NCI, Bethesda, MD), Agnès Vignery (Yale University, New Haven, CT), Theresa Whiteside (University of Pittsburgh, Pittsburgh, PA), Melissa Wong (Oregon Health and Science University, Portland, OR), Huang-Ge Zhang (University of Louisville, Louisville, KY).

References

1. Duelli D, Lazebnik Y. Cell-to-cell fusion as a link between viruses and cancer. Nat Rev Cancer 2007; 7:968-76. PMID: 18034186; DOI: 10.1038/nrc2272.
2. Cui W, Cuartas E, Ke J, Zhang Q, Einarsson HB, Sedgwick JD, et al. CD200 and its receptor, CD200R, modulate bone mass via the differentiation of osteoclasts. Proc Natl Acad Sci USA 2007; 104:14436-41. PMID: 17726108; DOI: 10.1073/pnas.0702811104.
3. Kukreja A, Radfar S, Sun BH, Insogna K, Dhodapkar MV. Dominant role of CD47-thrombospondin 1 interactions in myeloma induced fusion of human dendritic cells: implications for bone disease. Blood 2009; 114:3413-21. PMID: 19661269; DOI: 10.1182/blood-2009-03-211920.
4. Powell AE, Anderson EC, Davies PS, Silk AD, Pelz C, Impey S, et al. Fusion between Intestinal epithelial cells and macrophages in a cancer context results in nuclear reprogramming. Cancer Res 2011; 71:1497-505. PMID: 21303980; DOI: 10.1158/0008-5472.CAN-10-3223.
5. Lu X, Lu X, Kang Y. Organ-specific enhancement of metastasis by spontaneous ploidy duplication and cell size enlargement. Cell Res 2010; 20:1012-22. PMID: 20603645; DOI: 10.1038/cr.2010.93.
6. Skog J, Würdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nat Cell Biol 2008; 10:1470-6. PMID: 19011622; DOI: 10.1038/ncb1800.
7. Muralidharan-Chari V, Clancy J, Plou C, Romao M, Chavrier P, Raposo G, et al. ARF6-regulated shedding of tumor cell-derived plasma membrane microvesicles. Curr Biol 2009; 19:1875-85. PMID: 19896381 DOI: 10.1016/j.cub.2009.09.059.
8. Ghosh AK, Secreto CR, Knox TR, Ding W, Mukhopadhyay D, Kay NE. Circulating microvesicles in B-cell chronic lymphocytic leukemia can stimulate marrow stromal cells: implications for disease progression. Blood 2010; 115:1755-64. PMID: 20018914; DOI: 10.1182/blood-2009-09-242719.
9. Subra C, Grand D, Laulagnier K, Stella A, Lambeau G, Paillasse M, et al. Exosomes account for vesicle-mediated transcellular transport of activatable phospholipases and prostaglandins. J Lipid Res 2010; 51:2105-20. PMID: 20424270; DOI: 10.1194/jlr.M003657.
10. Szajnik M, Czystowska M, Szczepanski MJ, Mandapathil M, Whiteside TL. Tumor-derived microvesicles induce, expand and up-regulate biological activities of human regulatory T cells (Treg). PLoS ONE 2010; 5:e11469. PMID: 20661468; DOI: 10.1371/journal.pone.0011469.
11. Zhang H-G, Grizzle WE. Exosomes and cancer: A newly described pathway of immune suppression. Clin Cancer Res 2011; 17:959-64. PMID: 21224375; DOI: 10.1158/1078-0432.CCR-10-1489.