Year of publication 2019


**Solid stress in brain tumours causes neuronal loss and neurological dysfunction and can be reversed by lithium.**

_Nature biomedical engineering_ : 230-245 : [DOI : 10.1038/s41551-018-0334-7](https://doi.org/10.1038/s41551-018-0334-7)

**Summary**

The compression of brain tissue by a tumour mass is believed to be a major cause of the clinical symptoms seen in patients with brain cancer. However, the biological consequences of these physical stresses on brain tissue are unknown. Here, via imaging studies in patients and by using mouse models of human brain tumours, we show that a subgroup of primary and metastatic brain tumours, classified as nodular on the basis of their growth pattern, exert solid stress on the surrounding brain tissue, causing a decrease in local vascular perfusion as well as neuronal death and impaired function. We demonstrate a causal link between solid stress and neurological dysfunction by applying and removing cerebral compression, which respectively mimic the mechanics of tumour growth and of surgical resection. We also show that, in mice, treatment with lithium reduces solid-stress-induced neuronal death and improves motor coordination. Our findings indicate that brain-tumour-generated solid stress impairs neurological function in patients, and that lithium as a therapeutic intervention could counter these effects.

---

John D Martin, Giorgio Seano, Rakesh K Jain (2019 Feb 12)

**Normalizing Function of Tumor Vessels: Progress, Opportunities, and Challenges.**


**Summary**

Abnormal blood and lymphatic vessels create a hostile tumor microenvironment characterized by hypoxia, low pH, and elevated interstitial fluid pressure. These abnormalities fuel tumor progression, immunosuppression, and treatment resistance. In 2001, we proposed a novel hypothesis that the judicious use of antiangiogenesis agents—originally developed to starve tumors—could transiently normalize tumor vessels and improve the outcome of anticancer drugs administered during the window of normalization. In addition to providing preclinical and clinical evidence in support of this hypothesis, we also revealed the underlying molecular mechanisms. In parallel, we demonstrated that desmoplasia could also impair vascular function by compressing vessels, and that normalizing the extracellular matrix could improve vascular function and treatment outcome in both preclinical and clinical settings. Here, we summarize the progress made in
understanding and applying the normalization concept to cancer and outline opportunities and challenges ahead to improve patient outcomes using various normalizing strategies.

Year of publication 2018


A Glial Signature and Wnt7 Signaling Regulate Glioma-Vascular Interactions and Tumor Microenvironment.

Cancer cell : DOI: 10.1016/j.ccell.2018.03.020

Summary

Gliomas comprise heterogeneous malignant glial and stromal cells. While blood vessel co-option is a potential mechanism to escape anti-angiogenic therapy, the relevance of glial phenotype in this process is unclear. We show that Olig2 oligodendrocyte precursor-like glioma cells invade by single-cell vessel co-option and preserve the blood-brain barrier (BBB). Conversely, Olig2-negative glioma cells form dense perivascular collections and promote angiogenesis and BBB breakdown, leading to innate immune cell activation. Experimentally, Olig2 promotes Wnt7b expression, a finding that correlates in human glioma profiling. Targeted Wnt7a/7b deletion or pharmacologic Wnt inhibition blocks Olig2 glioma single-cell vessel co-option and enhances responses to temozolomide. Finally, Olig2 and Wnt7 become upregulated after anti-VEGF treatment in preclinical models and patients. Thus, glial-encoded pathways regulate distinct glioma-vascular microenvironmental interactions.

Hadi T Nia, Meenal Datta, Giorgio Seano, Peigen Huang, Lance L Munn, Rakesh K Jain (2018 Apr 21)

Quantifying solid stress and elastic energy from excised or in situ tumors.


Summary

Solid stress, distinct from both tissue stiffness and fluid pressure, is a mechanical stress that is often elevated in both murine and human tumors. The importance of solid stress in tumor biology has been recognized in initial studies: solid stress promotes tumor progression and lowers the efficacy of anticancer therapies by compressing blood vessels and contributing to hypoxia. However, robust, reproducible, and objective methods that go beyond demonstration and bulk measurements have not yet been established. We have developed
three new techniques to rigorously measure and map solid stress in both human and murine tumors that are able to account for heterogeneity in the tumor microenvironment. We describe here these methods and their independent advantages: 2D spatial mapping of solid stress (planar-cut method), sensitive estimation of solid stress in small tumors (slicing method), and in situ solid-stress quantification (needle-biopsy method). Furthermore, the preservation of tissue morphology and structure allows for subsequent histological analyses in matched tumor sections, facilitating quantitative correlations between solid stress and markers of interest. The three procedures each require ~2 h of experimental time per tumor. The required skill sets include basic experience in tumor resection and/or biopsy (in mice or humans), as well as in intravital imaging (e.g., ultrasonography).


**Lymph node metastases can invade local blood vessels, exit the node, and colonize distant organs in mice.**


**Summary**

Lymph node metastases in cancer patients are associated with tumor aggressiveness, poorer prognoses, and the recommendation for systemic therapy. Whether cancer cells in lymph nodes can seed distant metastases has been a subject of considerable debate. We studied mice implanted with cancer cells (mammary carcinoma, squamous cell carcinoma, or melanoma) expressing the photoconvertible protein Dendra2. This technology allowed us to selectively photoconvert metastatic cells in the lymph node and trace their fate. We found that a fraction of these cells invaded lymph node blood vessels, entered the blood circulation, and colonized the lung. Thus, in mouse models, lymph node metastases can be a source of cancer cells for distant metastases. Whether this mode of dissemination occurs in cancer patients remains to be determined.

Giorgio Seano (2017 Nov 15)

**Targeting the perivascular niche in brain tumors.**

*Current opinion in oncology* : 54-60 : [DOI : 10.1097/CCO.0000000000000417]

**Summary**

Brain tumors are composed of primary tumors of the central nervous system, such as glioblastoma (GBM), and secondary metastatic tumors, such as melanoma, non-Hodgkin lymphoma as well as lung and breast cancers. Brain tumors are highly deadly, and unfortunately not many improvements have been achieved to improve the survival of patients with brain tumors. Chemoradiation resistance is one of the most clinically relevant challenges faced in patients with brain tumors. The perivascular niche is one of the most relevant microenvironment hubs in brain tumors. The understanding of the cellular crosstalk...
established within the brain tumor perivascular niche might provide us with key discoveries of new brain tumor vulnerabilities.

**Year of publication 2017**

Despina Bazou, Nir Maimon, Gabriel Gruionu, Jelena Grahovac, Giorgio Seano, Hao Liu, Conor L Evans, Lance L Munn (2017 Jun 2)

**Vascular beds maintain pancreatic tumour explants for ex vivo drug screening.**

*Journal of tissue engineering and regenerative medicine* : DOI: 10.1002/term.2481

**Summary**

Our understanding of cancer progression or response to therapies would benefit from benchtop, tissue-level assays that preserve the biology and anatomy of human tumours ex vivo. We present a methodology for maintaining patient tumour samples ex vivo for the purpose of drug testing in a clinical setting. The harvested tumour biopsy, excised from mice or patients, is integrated into a support tissue that includes stroma and vasculature. This support tissue preserves tumour histoarchitecture and relevant expression profiles, and tumour tissues cultured using this system display different sensitivities to chemotherapeutics compared with tumour explants with no supporting tissue. The methodology is more rapid than patient-derived xenograft models, easy to implement, and amenable to high-throughput assays, making it an attractive tool for in vitro drug screening or for the guidance of patient-specific chemotherapies.

**Year of publication 2016**

Giorgio Seano, Luca Primo (2016 May 14)

**Human Arterial Ring Angiogenesis Assay.**


**Summary**

In this chapter we describe a model of human angiogenesis where artery explants from umbilical cords are embedded in gel matrices and subsequently produce capillary-like structures. The human arterial ring (hAR) assay is an innovative system that enables three-dimensional (3D) and live studies of human angiogenesis. This ex vivo model has the advantage of recapitulating several steps of angiogenesis, including endothelial sprouting, migration, and differentiation into capillaries. Furthermore, it can be exploited for (1) identification of new genes regulating sprouting angiogenesis, (2) screening for pro- or anti-angiogenic drugs, (3) identification of biomarkers to monitor the efficacy of anti-angiogenic regimens, and (4) dynamic analysis of tumor microenvironmental effects on vessel formation.
Ang-2/VEGF bispecific antibody reprograms macrophages and resident microglia to anti-tumor phenotype and prolongs glioblastoma survival. 

Proceedings of the National Academy of Sciences of the United States of America: 4476-81: DOI: 10.1073/pnas.1525360113

Summary

Inhibition of the vascular endothelial growth factor (VEGF) pathway has failed to improve overall survival of patients with glioblastoma (GBM). We previously showed that angiopoietin-2 (Ang-2) overexpression compromised the benefit from anti-VEGF therapy in a preclinical GBM model. Here we investigated whether dual Ang-2/VEGF inhibition could overcome resistance to anti-VEGF treatment. We treated mice bearing orthotopic syngeneic (Gl261) GBMs or human (MGG8) GBM xenografts with antibodies inhibiting VEGF (B20), or Ang-2/VEGF (CrossMab, A2V). We examined the effects of treatment on the tumor vasculature, immune cell populations, tumor growth, and survival in both the Gl261 and MGG8 tumor models. We found that in the Gl261 model, which displays a highly abnormal tumor vasculature, A2V decreased vessel density, delayed tumor growth, and prolonged survival compared with B20. In the MGG8 model, which displays a low degree of vessel abnormality, A2V induced no significant changes in the tumor vasculature but still prolonged survival. In both the Gl261 and MGG8 models A2V reprogrammed protumor M2 macrophages toward the antitumor M1 phenotype. Our findings indicate that A2V may prolong survival in mice with GBM by reprogramming the tumor immune microenvironment and delaying tumor growth.

Dual inhibition of Ang-2 and VEGF receptors normalizes tumor vasculature and prolongs survival in glioblastoma by altering macrophages. 

Proceedings of the National Academy of Sciences of the United States of America: 4470-5: DOI: 10.1073/pnas.1525349113

Summary

Glioblastomas (GBMs) rapidly become refractory to anti-VEGF therapies. We previously demonstrated that ectopic overexpression of angiopoietin-2 (Ang-2) compromises the
benefits of anti-VEGF receptor (VEGFR) treatment in murine GBM models and that circulating Ang-2 levels in GBM patients rebound after an initial decrease following cediranib (a pan-VEGFR tyrosine kinase inhibitor) administration. Here we tested whether dual inhibition of VEGFR/Ang-2 could improve survival in two orthotopic models of GBM, GL261 and U87. Dual therapy using cediranib and MEDI3617 (an anti-Ang-2-neutralizing antibody) improved survival over each therapy alone by delaying GL261 growth and increasing U87 necrosis, effectively reducing viable tumor burden. Consistent with their vascular-modulating function, the dual therapies enhanced morphological normalization of vessels. Dual therapy also led to changes in tumor-associated macrophages (TAMs). Inhibition of TAM recruitment using an anti-colony-stimulating factor-1 antibody compromised the survival benefit of dual therapy. Thus, dual inhibition of VEGFR/Ang-2 prolongs survival in preclinical GBM models by reducing tumor burden, improving normalization, and altering TAMs. This approach may represent a potential therapeutic strategy to overcome the limitations of anti-VEGF monotherapy in GBM patients by integrating the complementary effects of anti-Ang2 treatment on vessels and immune cells.

Year of publication 2015

Sara M Tolaney, Yves Boucher, Dan G Duda, John D Martin, Giorgio Seano, Marek Ancukiewicz, William T Barry, Shom Goel, Johanna Lahdenranta, Steven J Isakoff, Eren D Yeh, Saloni R Jain, Mehra Golsan, Jane Brock, Matija Snuderl, Eric P Winer, Ian E Krop, Rakesh K Jain (2015 Nov 19)

Role of vascular density and normalization in response to neoadjuvant bevacizumab and chemotherapy in breast cancer patients.
Proceedings of the National Academy of Sciences of the United States of America : 14325-30 :
DOI : 10.1073/pnas.1518808112

Summary

Preoperative bevacizumab and chemotherapy may benefit a subset of breast cancer (BC) patients. To explore potential mechanisms of this benefit, we conducted a phase II study of neoadjuvant bevacizumab (single dose) followed by combined bevacizumab and adriamycin/cyclophosphamide/paclitaxel chemotherapy in HER2-negative BC. The regimen was well-tolerated and showed a higher rate of pathologic complete response (pCR) in triple-negative (TN)BC (11/21 patients or 52%, [95% confidence interval (CI): 30,74]) than in hormone receptor-positive (HR)BC [5/78 patients or 6% (95%CI: 2,14)]. Within the HRBCs, basal-like subtype was significantly associated with pCR (P = 0.007; Fisher exact test). We assessed interstitial fluid pressure (IFP) and tissue biopsies before and after bevacizumab monotherapy and circulating plasma biomarkers at baseline and before and after combination therapy. Bevacizumab alone lowered IFP, but to a smaller extent than previously observed in other tumor types. Pathologic response to therapy correlated with sVEGFR1 postbevacizumab alone in TNBC (Spearman correlation 0.610, P = 0.0033) and pretreatment microvascular density (MVD) in all patients (Spearman correlation 0.465, P = 0.0005). Moreover, increased pericyte-covered MVD, a marker of extent of vascular normalization, after bevacizumab monotherapy was associated with improved pathologic response to treatment, especially in patients with a high pretreatment MVD. These data suggest that bevacizumab prunes vessels while normalizing those remaining, and thus is
beneficial only when sufficient numbers of vessels are initially present. This study implicates pretreatment MVD as a potential predictive biomarker of response to bevacizumab in BC and suggests that new therapies are needed to normalize vessels without pruning.

Vasileios Askoxylakis, Gino B Ferraro, David P Kodack, Mark Badeaux, Ram C Shankaraiah, Giorgio Seano, Jonas Kloepper, Trupti Vardam, John D Martin, Kamila Naxerova, Divya Bezwada, Xiaolong Qi, Martin K Selig, Elena Brachtel, Dan G Duda, Peigen Huang, Dai Fukumura, Jeffrey A Engelman, Rakesh K Jain (2015 Nov 9)

Preclinical Efficacy of Ado-trastuzumab Emtansine in the Brain Microenvironment.
*Journal of the National Cancer Institute*: DOI: [10.1093/jnci/djv313](http://dx.doi.org/10.1093/jnci/djv313)

**Summary**

Central nervous system (CNS) metastases represent a major problem in the treatment of human epidermal growth factor receptor 2 (HER2)-positive breast cancer because of the disappointing efficacy of HER2-targeted therapies against brain lesions. The antibody-drug conjugate ado-trastuzumab emtansine (T-DM1) has shown efficacy in trastuzumab-resistant systemic breast cancer. Here, we tested the hypothesis that T-DM1 could overcome trastuzumab resistance in murine models of brain metastases.

Alberto Puliafito, Alessandro De Simone, Giorgio Seano, Paolo Armando Gagliardi, Laura Di Blasio, Federica Chianale, Andrea Gamba, Luca Primo, Antonio Celani (2015 Oct 17)

Three-dimensional chemotaxis-driven aggregation of tumor cells.
*Scientific reports*: 15205 : DOI: [10.1038/srep15205](http://dx.doi.org/10.1038/srep15205)

**Summary**

One of the most important steps in tumor progression involves the transformation from a differentiated epithelial phenotype to an aggressive, highly motile phenotype, where tumor cells invade neighboring tissues. Invasion can occur either by isolated mesenchymal cells or by aggregates that migrate collectively and do not lose completely the epithelial phenotype. Here, we show that, in a three-dimensional cancer cell culture, collective migration of cells eventually leads to aggregation in large clusters. We present quantitative measurements of cluster velocity, coalescence rates, and proliferation rates. These results cannot be explained in terms of random aggregation. Instead, a model of chemotaxis-driven aggregation – mediated by a diffusible attractant – is able to capture several quantitative aspects of our results. Experimental assays of chemotaxis towards culture conditioned media confirm this hypothesis. Theoretical and numerical results further suggest an important role for chemotactic-driven aggregation in spreading and survival of tumor cells.

Paolo Armando Gagliardi, Alberto Puliafito, Laura di Blasio, Federica Chianale, Desiana Somale,
Giorgio Seano, Federico Bussolino, Luca Primo (2015 May 16)

**Real-time monitoring of cell protrusion dynamics by impedance responses.**

*Scientific reports*: 10206 : [DOI: 10.1038/srep10206]

**Summary**

Cellular protrusions are highly dynamic structures involved in fundamental processes, including cell migration and invasion. For a cell to migrate, its leading edge must form protrusions, and then adhere or retract. The spatial and temporal coordination of protrusions and retraction is yet to be fully understood. The study of protrusion dynamics mainly relies on live-microscopy often coupled to fluorescent labeling. Here we report the use of an alternative, label-free, quantitative and rapid assay to analyze protrusion dynamics in a cell population based on the real-time recording of cell activity by means of electronic sensors. Cells are seeded on a plate covered with electrodes and their shape changes map into measured impedance variations. Upon growth factor stimulation the impedance increases due to protrusive activity and decreases following retraction. Compared to microscopy-based methods, impedance measurements are suitable to high-throughput studies on different cell lines, growth factors and chemical compounds. We present data indicating that this assay lends itself to dissect the biochemical signaling pathways controlling adhesive protrusions. Indeed, we show that the protrusion phase is sustained by actin polymerization, directly driven by growth factor stimulation. Contraction instead mainly relies on myosin action, pointing at a pivotal role of myosin in lamellipodia retraction.

---

Giorgio Seano, Luca Primo (2015 Mar 20)

**Podosomes and invadopodia: tools to breach vascular basement membrane.**

*Cell cycle (Georgetown, Tex.)* : 1370-4 : [DOI: 10.1080/15384101.2015.1026523]

**Summary**

The vascular basement membrane (BM) is a thin and dense cross-linked extracellular matrix layer that covers and protects blood vessels. Understanding how cells cross the physical barrier of the vascular BM will provide greater insight into the potentially critical role of vascular BM breaching in cancer extravasation, leukocyte trafficking and angiogenic sprouting. In the last year, new evidence has mechanistically linked the breaching of vascular BM with the formation of specific cellular micro-domains known as podosomes and invadopodia. These structures are specialized cell-matrix contacts with an inherent ability to degrade the extracellular matrix. Specifically, the formation of podosomes or invadopodia was shown as an important step in vascular sprouting and tumor cell extravasation, respectively. Here, we review and comment on these recent findings and explore the functions of podosomes and invadopodia within the context of pathological processes such as tumor dissemination and tumor angiogenesis.