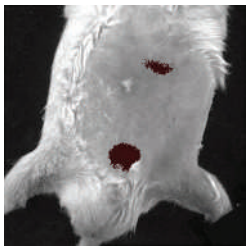
Breaking Advances
Highlights from Recent Cancer Literature

Ex vivo Imaging of Lymphatics



Clinical and experimental studies have revealed the importance of lymphatic vasculature for regulating aspects of early- and late-stage cancer progression, as well as postsurgical lymphedema, that plague many patients. Clinical approaches for imaging lymphatics are now emerging. Proulx and colleagues have developed a novel liposomal (LP) formulation of indocyanine green (ICG), a near-infrared (NIR) dye, to visualize deep lymph nodes and quantitatively evaluate lymphatic function that has been approved by the FDA for examining hepatic function and cardiac output and for ophthalmic angiography. When injected intradermally in mice, LP-ICG was specifically taken up by lymphatic vessels, allowing improved visualization of deep lymph nodes. In mice bearing B16 luciferase-expressing melanomas, sequential NIR imaging of intradermally injected LP-ICG also enabled quantification of lymphatic flow that varied as a function of lymph node tumor burden. ICG is less stable than LP-ICG in solution and rapidly enters venous capillaries after local injection; thus, the novel formulation of LP-ICG has improved stability compared with ICG in solution, allowing increased fluorescence signal with a shift toward longer wavelength absorption and emission. These improvements will facilitate quantitative studies of lymphatic function *in vivo* for preclinical investigations and may enable imaging of lymphedema or improved sentinel lymph node detection in cancer patients. (*Image from cited article courtesy of publisher.*)

Proulx ST, Luciani P, Derzsi S, et al. Quantitative imaging of lymphatic function with liposomal indocyanine green. Can Res 2010;18:7053–62.

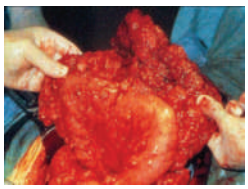
Dendritic Cells and Induction of Th2 Immunity

Dendritic cells (DCs) are a diverse subset of professional antigen presenting cells (APC) pre-stationed in tissues where they facilitate rapid responses to tissue damage through their ability to prime and direct T-cell function. Whereas the importance of DCs for directing Th1-type immune responses against a variety of viral and bacterial pathogens has been clear for some time, their ability to similarly direct Th2-type responses following infection has lacked mechanistic clarity. Two studies evaluating DCs as regulators of Th2 immunity provide insight into these issues. Phythian-Adams and colleagues found that $CD11c^+$ DCs, and not basophils, were significant for priming Th2 $CD4^+$ T-cell responses following parasitic helminth infection. Hammad and colleagues identified $CD11b^+$ $Fc\varepsilon RI^+$ DCs as significant for initiating Th2 responses following exposure to household allergens associated with asthma. Depletion of these distinct DC subsets in each model similarly altered the cytokine microenvironment to favor Th1 cytokine production. While these studies highlight the significance and diversity of DC subtypes involved in regulating important tissue responses, they also raise the intriguing possibility that in cancers regulated by Th2 cytokines/cells, similar differences must be examined, especially when considering DC-based vaccine strategies where tissue/microenvironment specificity will likely be critical for response.

Phythian-Adams AT, Cook PC, Lundie RJ, et al. CD11c depletion severely disrupts Th2 induction and development in vivo. J Exp Med 2010 Sept 6 [Epub ahead of print].

Hammad H, Plantinga M, Deswarte K, et al. Inflammatory dendritic cells—not basophils—are necessary and sufficient for induction of Th2 immunity to inhaled house dust mite allergen. J Exp Med 2010 Sept 7 [Epub ahead of print].

Serum Assays for Early Cancer Detection: Have They Come of Age?



A major goal for cancer prevention is generation of inexpensive and reliable assays that detect early-stage malignancy. Two recent studies report results using different serum bioassays for detection of ovarian cancer and pancreatic adenocarcinoma, two cancer types that are difficult to diagnose and in which late-stage detection is the norm. Zhou and colleagues used high-throughput ambient ionization mass spectrometry for metabolic profiling of blood sera to distinguish between cancer and control groups (healthy women or women with benign conditions) with an astonishing 99% to 100% accuracy. Using a serum immunoassay and monoclonal antibody PAM4, reactive with a unique biomarker expressed by greater than 85% of pancreatic adenocarcinomas, Gold and colleagues detected pancreatic adenocarcinoma with 82% sensitivity. Importantly, PAM4 revealed patients with advanced disease based on significantly higher antigen levels than those with early-stage disease and identified evidence of neoplastic precursor lesions in serum-positive pancreatitis patients. Both studies provide compelling proof for the use of these assays as diagnostic tools for high-risk groups and possibly for monitoring cancer recurrence after therapeutic intervention. If these assays are employed as routine diagnostic assays, positive results would motivate early therapeutic intervention that would likely improve patient outcomes for both of these cancer types. (*Image courtesy of E. Lengyel, University of Chicago, Chicago IL.*)

Zhou M, Guan W, Walker LD, et al. Rapid mass spectrometric metabolic profiling of blood sera detects ovarian cancer with high accuracy. Cancer Epidemiol Biomarkers Prev 2010;19:2262–71.

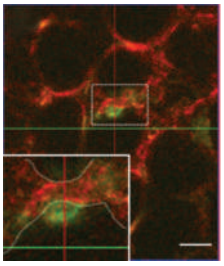
Gold DV, Goggins M, Modrak DE, et al. Detection of early-stage pancreatic adenocarcinoma. Cancer Epidemiol Biomarkers Prev; Published OnlineFirst September 1, 2010; doi:10.1158/1055-9965.EPI-10-0667.

Two Ligands for CSF-1R

Macrophages have emerged as significant paracrine regulators of solid tumor development based on their ability to regulate tissue remodeling, hypoxia and angiogenesis, T-cell function, epithelial proliferation, invasion, and metastasis. Macrophage colony stimulating factor-1 (CSF-1) has long been known to represent the primary regulator of macrophage survival, proliferation, and differentiation, mediated by the high affinity protein tyrosine kinase CSF-1 receptor (c-fms). As such, several pharmaceutical manufacturers have developed antagonists targeting either the ligand or receptor with the hope of generating a potent anticancer therapeutic that dismantles macrophage-mediated potentiation of carcinogenesis. *CSF1*$^{-/-}$ and *Csf1*$^{op/op}$ mice display essentially identical defects, however, severity of defects in *CSF1r*$^{-/-}$ mice vary as a function of strain, suggesting the existence of a second ligand, recently identified as interleukin (IL)-34. Whereas both ligands are functionally conserved across vertebrates, their redundant and non-redundant roles as regulators of macrophage function via CSF-1R are unknown. Wei and colleagues evaluated expression of *IL34*, as compared to *CSF1* in mice by *in situ* hybridization, and report that *IL34* mRNA is expressed in embryonic brain at E11.5, prior to expression of *Csf1* mRNA. Quantitative real-time PCR revealed that, as compared with *Csf1* mRNA, *IL34* mRNA levels were lower in pregnant uterus and in cultured osteoblasts but higher in brain and heart, supporting the argument that the varied spatiotemporal expression of IL-34 and CSF-1 may facilitate complementary activation of CSF-1R in developing and adult tissues. If similar variability in ligand regulation is at play in solid tumors, it is reasonable to anticipate varied results examining the anticancer efficacy of drugs targeting the ligand, as compared with those blocking the kinase activity of the receptor.

Wei S, Nandi S, Chitu V, et al. Functional overlap but differential expression of CSF-1 and IL-34 in their CSF-1 receptor-mediated regulation of myeloid cells. J Leukoc Biol 2010;88:495–505.

Ex vivo Imaging of Liver and Lung Metastasis



Sites of productive cancer metastasis are regulated not only by intrinsic parameters of malignant cells but also by extrinsic forces influencing malignant cell behavior in ectopic tissues. Breast cancers metastasize to multiple secondary sites, including liver and lung, but kinetics and frequency of metastatic growth in these sites vary. Moreover, while liver metastasis is more frequent in women with breast cancer, experimental studies have instead been reliant on murine models of mammary carcinogenesis where lung metastasis predominates; thus, information regarding kinetics and parameters of liver metastasis remain scant. Using *ex vivo* two-photon microscopy, Martin and colleagues compared murine mammary adenocarcinoma cell extravasation rates in liver and lung to reveal that malignant cell extravasation was favored in liver parenchyma as opposed to lung where a large fraction of malignant cells instead remained within the vasculature and formed intravascular "metastatic" colonies. This study articulates the need for development of experimental models more representative of human cancer, as well as more routine use of state-of-the-art imaging modalities for their validation.
(*Image from cited article courtesy of publisher.*)

Martin MD, Kremers GJ, Short KW, et al. Rapid extravasation and establishment of breast cancer micrometastases in the liver microenvironment. Mol Cancer Res; Published OnlineFirst August 19, 2010; doi:10.1158/1541-7786.MCR-09-0551.

V600E *BRAF*+ Melanoma: Results from Phase I Study Engender Optimism

Forty to 60% of melanomas, and 7 to 8% of all cancers, carry an activating mutation in the gene encoding the serine-threonine protein kinase BRAF, 90% of which represent the V600E mutation that constitutively activates BRAF and the mitogen activated protein kinase pathway. In two recent publications, Flaherty and colleagues and Bollag and colleagues report on results from a multicenter phase I dose-escalation trial evaluating PLX4032, an orally available inhibitor of mutated *BRAF*. Fifty-five patients (49 with melanoma) were enrolled in the dose-escalation phase, and 32 additional patients with metastatic melanoma harboring the V600E *BRAF* mutation were enrolled in the extension phase. The majority of patients treated with PLX4032 exhibited complete or partial tumor regression with an estimated median progression-free survival of more than 7 months, thus demonstrating the degree to which *BRAF*-mutant melanomas are BRAF kinase–dependent.

Flaherty KT, Puzanov I, Kim KB, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. N Engl J Med 2010; 363:809–19.

Bollag G, Hirth P, Tsai J. Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. Nature 2010 Sep 7 [Epub ahead of print].

Note: *Breaking Advances* are written by *Cancer Research* Editors. Readers are encouraged to consult the articles referred to in each item for full details on the findings described.