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# INSIDE LAB INVEST

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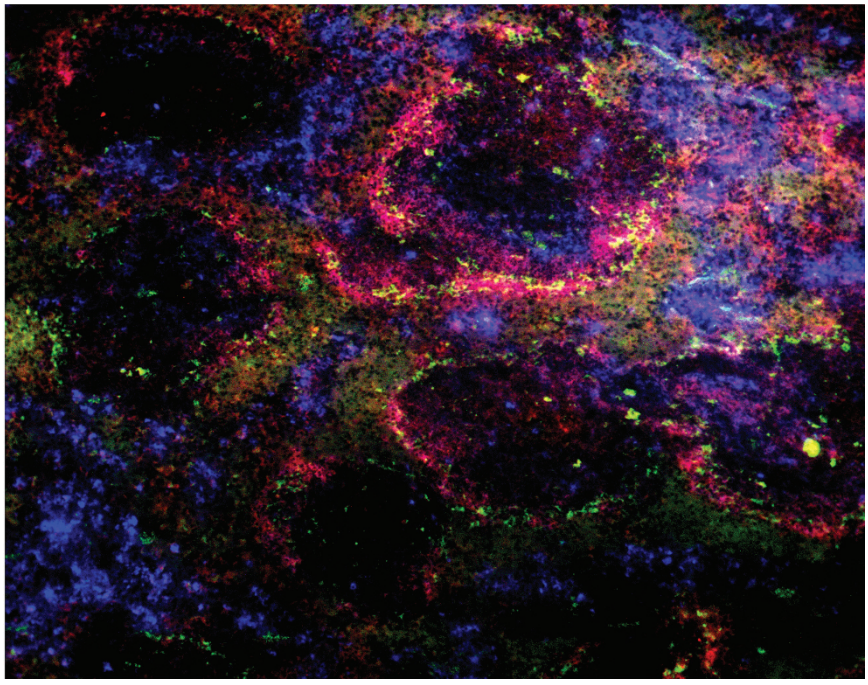

## Intrafollicular CD1d$^{hi}$/MZ B cells in lupus mice

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Marginal-zone (MZ) B cells are potent T-cell activators that respond more rapidly than follicular B cells to antigen to differentiate rapidly into plasma cells, and they play an essential role in transporting antigens to the follicle. In addition, MZ B cells respond better to T-cell signals than do follicular B cells *in vitro* but not *in vivo*, indicating that anatomical and physiological barriers prevent *in vivo* activation of MZ B cells. These observations have led to the hypothesis that sequestration of MZ B cells from the follicles in the MZ area where very few T cells are located represents a B-cell tolerance checkpoint. The expansion of the MZ B-cell compartment has been directly implicated in lupus pathogenesis in some mouse models. However, the cells' involvement through altered functions or location has not been assessed.

Duan *et al* have found that in B6.TC lupus-prone mice a large proportion of MZ B cells are located outside the MZ and inside the follicles, and that these B6.TC MZ B cells are more activated and readily differentiated than B6 MZ Bs. Furthermore, the restoration of MZ B-cell follicular exclusion by B7-2 deficiency correlates with a significant reduction in autoimmune pathology in B6.TC mice. Overall, these results strongly suggest that a breach in MZ B-cell follicular exclusion plays a significant role in lupus pathogenesis in the B6.TC model. In addition to the molecular mechanisms responsible for this breach in follicular exclusion, a critical issue that needs to be addressed is whether autoreactive B cells are preferentially involved in this process.

## A role for VEGF in the inflammation of diabetic nephropathy

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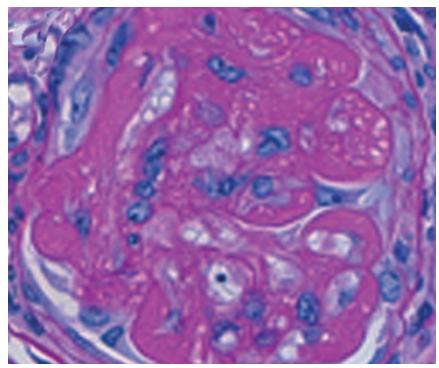
Loss of kidney function is one of the key life-limiting features of diabetes. Sustained exposure of the glomerulus to high serum levels of glucose and advanced glycation end products leads to extensive renal vascular disease and a convergence of oxidative and inflammatory injury. In the last instance, induction of intercellular adhesion molecule-1 and monocyte chemoattractant protein-1 in the kidney is hypothesized to stimulate leukocyte infiltration into the glomerulus. Increased renal expression of vascular endothelial growth factor (VEGF) may also provoke glomerular damage through the uncoupling of endothelial nitric oxide synthase (eNOS). Sato *et al* tested both hypotheses by examining influx of macrophages into the glomeruli of diabetic eNOS-deficient mice. When compared with diabetic wild-type mice, the diabetic eNOS-deficient mice exhibited markedly increased glomerular macrophage infiltrate associated with increased podocyte VEGF expression. In *in vitro* experiments, nitric oxide was shown to block macrophage migration and hypertrophy, in part by downregulating Flt-1 expression on the macrophage. These findings implicate VEGF-induced uncoupling of eNOS generation as a potentiating factor in the inflammatory component of diabetic nephropathy.



## Epigenetic silencing of CST6 in gliomas

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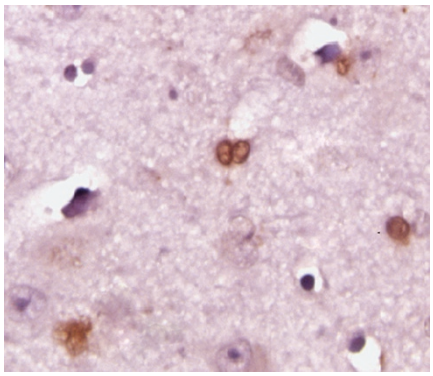
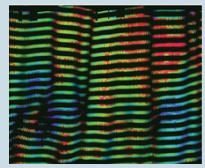
Repression of gene expression by promoter hypermethylation is a putative mechanism of tumorigenesis. The *CST6* gene encodes cystatin E/M, which is a potent inhibitor of cathepsins B, L, H, and V (lysosomal proteases). *CST6* expression is frequently downregulated epigenetically



in breast carcinomas, and this is believed to promote tissue invasion and metastasis by loss of protease inhibition. Proteases also play a role in the highly infiltrative biologic behavior of diffuse gliomas, which include astrocytoma, oligodendroglioma, and glioblastoma (the most common primary malignant brain tumor in adults). Cathepsin B is frequently overexpressed by infiltrating gliomas and is known to be strongly inhibited by cystatin E/M.

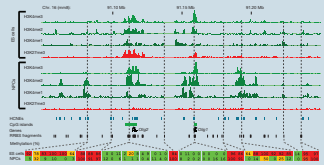
Qiu *et al* show that *CST6* is normally expressed in astrocytes and oligodendroglia but is downregulated in human gliomas. Using both methylation-specific PCR and pyrosequencing methods, the authors found a high frequency of *CST6* promoter hypermethylation that strongly correlated with cystatin E/M negativity by immunolabeling. No promoter methylation was found in normal brain, which suggests that *CST6* hypermethylation is tumor-specific. *In vitro* studies then revealed that *CST6* is expressed and hypomethylated in neural stem cells and that the gene is markedly induced when these cells differentiate. Furthermore, *CST6* was hypermethylated and silenced in a glioma tumor stem cell line in which differentiation-induced upregulation was also blocked, suggesting that *CST6* methylation may be an early event in tumorigenesis. *CTS6* expression suppressed motility and invasiveness of glioma cells in culture. These results support an epigenetic mechanism of *CST6* gene silencing in the pathogenesis of infiltrating glial neoplasms.

# nature.com/pathology



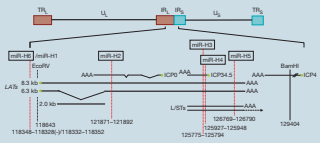
**Lights, camera, actin!** Analysis of muscle biopsies is a highly specialized process. Although the variety of staining reactions used provides important insights into muscle function—and dysfunction—the physical nature of histologic sections prevents analysis of entire sarcomeres. Thus, pathologists and neurologists must infer sarcomere contractility by integrating clinical data, electrophysiologic analysis, and histopathology. A new microendoscope-based imaging technique has permitted the direct visualization of individual sarcomeres and their dynamic length variations in living mice and humans. This technique provides insight into the spatial arrangement of sarcomeres and biomechanics of muscle function. As this approach is applied to human disease, it has the potential to complement available tools for diagnosing neuromuscular disease and monitoring response to therapy.

*Nature*, published online 6 July 2008; doi:10.1038/nature07104



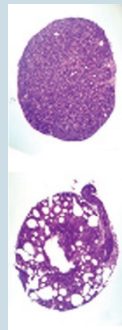
**Crystallizing methylation: a new perspective on epigenomics** The availability of the human genome sequence and relatively inexpensive high-throughput tools for sequencing DNA specimens has advanced our understanding of many diseases at a remarkable pace. However, diseases that result from epigenomic events have been more difficult to unravel. A letter recently published in *Nature* reports a novel method for assessing the distribution of DNA methylation throughout the genome. The results indicate that DNA methylation is highly dynamic and that DNA methylation patterns are more closely related to histone methylation and cell state than to genome sequence. For example, cell differentiation is associated with extensive changes in methylation, particularly in regulatory regions near core promoters. Extended proliferation is marked by the aberrant hypermethylation of CpG islands associated with developmentally regulated genes in a pattern reminiscent of some tumors. This new tool for genome-wide examination of DNA methylation may allow the determination of molecular signatures that explain the unique morphologies associated with disease.

*Nature*, published online 6 July 2008; doi:10.1038/nature07107



**MicroRNAs cause herpes to hide out** It is well known that herpesviruses can be latent in an individual for decades before they cause new disease. Herpes zoster, which causes chicken pox at first infection, can re-emerge later. A recent study in *Nature* sheds light on the genetic regulatory elements that allow herpes simplex virus 1 to hide out in neurons. The prevailing genetic product of the latent state, the unstable latency-associated transcript (*LAT*), is a precursor for four distinct microRNAs that counteract the expression of viral proteins required for active infection. These data provide clues to new means of preventing debilitating viral reactivation in infected individuals and may also help to limit disease transmission.

*Nature*, published online 2 July 2008; doi:10.1038/nature07103



**Don't be so inflammatory!** End-stage renal disease is frequently associated with cyst formation, although generally not as extensive as that seen in polycystic kidney disease. A recent study reported in *Nature Medicine* may explain this phenomenon. The work shows that tumor necrosis factor-α within cysts of patients with autosomal dominant polycystic kidney disease (ADPKD) promotes cystogenesis by disrupting the targeting of polycystin-2 to the plasma membrane and primary cilia. Consistent with this, exogenous TNF caused cyst formation in wild-type mice and accelerated cyst formation in mice heterozygous for polycystin-2 (*Pkd2*), one of two genes responsible for the bulk of human ADPKD. Conversely, etanercept, a TNF inhibitor, prevented cyst formation in $Pkd2^{+/-}$ mice. These data establish a new role for TNF in cyst formation and may pave the way for new therapies to prevent cyst formation and growth in individuals with ADPKD.

*Nature Medicine*, published online 15 June 2008; doi:10.1038/nm1783