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doi:10.1038/labinvest.2013.68

Repairing DNA for molecular analysis

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Most pathology specimens are stored as formalin-fixed paraffin-embedded (FFPE) tissues or glass slides. Millions of such specimens are available for study in pathology department archives. Because FFPE tissues contain nucleic acids, they potentially can be studied using modern genomic technologies. However, their RNA and DNA is often degraded and chemically modified, and are worse over time—it is very difficult to study nucleic acids from FFPE tissue that has been stored for more than 10 years.

To address the problem of DNA degradation associated with this process, quality control steps have been suggested. In addition, several commercial methods are available that improve the quality of the DNA by enzymatically repairing overhangs and gaps in degraded DNA and adducts in chemically modified sequences and by ligating short DNA fragments to produce longer ones.

In light of these recent developments, Hosein *et al* assessed the effectiveness of DNA 'restoration' as a pretreatment step prior to single-nucleotide polymorphism (SNP)–chromosomal genomic hybridization analysis of DNA from FFPE tissue, including tissues processed as much as 50 years previously. They found that quality control evaluation via qPCR was able to predict the samples that would be improved by DNA restoration.

With samples for which the quality control values predicted a low level of success, the authors found that the DNA-restoration step significantly improved the performance of FFPE DNA, generating higher SNP call rates and reduced 'noise.' This study shows that developments to improve the quality of DNA from FFPE tissue are leading to improvements in our ability to salvage FFPE tissues for modern genomic technologies.

CD30-mediated cell death in ALCL lymphoma

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CD30, a member of the tumor necrosis factor receptor (TNFR) family, is ubiquitously expressed on the surface of anaplastic large-cell lymphoma (ALCL) cells. It is also expressed on the surface of Hodgkin and Reed Sternberg cells in classic Hodgkin lymphoma. Its expression in normal, nonneoplastic cells is restricted to subsets of activated B and T cells. Therefore, owing to its restricted expression in ALCL and Hodgkin lymphoma

cells, CD30 is an attractive pharmacological target. Yet it has been difficult to determine how to target CD30. Its induction in ALCL cells results in cell-cycle arrest but not cell death. Interestingly, if nuclear factor (NF- κ B) is inhibited concurrently with CD30 stimulation, then ALCL cells die. It is unclear how CD30 activation stimulates cell death in this setting.

Hirsch *et al* made use of CD30's ability to stimulate cell death when NF- κ B is inhibited in order to investigate this mechanism with the overall goal of exploiting it therapeutically. They determined that receptor-interacting protein 1 (RIP1), a death homology domain–containing serine/threonine kinase, forms a complex with CD30 in NF- κ B-inhibited ALCL cells to stimulate cell death. The actual mechanism of RIP1 stimulated cell death in this setting appeared to have hybrid features of both caspase-dependent apoptosis and necroptosis, a form of programmed necrosis that is dependent on RIP1. Because compounds such as bortezomib, a proteasome inhibitor, are effective NF- κ B inhibitors, these results suggest that a combination of bortezomib and CD30 stimulation by a therapeutic anti-CD30 antibody merit consideration for treatment of ALCL cells that express RIP1.

Regulation of perivascular supporting cells by Cyp1B1

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Angiogenesis requires the coordinated efforts of both the endothelial cells (ECs) that line blood vessels and the cells that support endothelial cells, such as pericytes (PCs) and

smooth muscle cells. PCs are involved in the synthesis, remodeling, and maintenance of the vascular basement membrane, local regulation of vascular tone, and promotion of vascular stability. Abnormal PC function has been linked to diabetic retinopathy, neonatal intraventricular hemorrhage, cancers, neurodegenerative disorders, and other diseases.

It has recently been shown that retinas from mice deficient in cytochrome P4501B1 (Cyp1b1) exhibit reduced vascular density and fail to undergo neovascularization during oxygen-induced ischemic retinopathy. Examination of the endothelial cells from these mice demonstrated a variety of defects. In further investigating the vascular defects found in these mice, Palenski *et al* found that PCs constitutively expressed Cyp1b, suggesting that it plays a role in regulating oxidation levels in PCs. PCs from Cyp1b1^{-/-} knockout mice had higher reactive oxygen species levels and numerous defects related to increased oxidative stress. They also exhibited defects in proliferation and migration and interactions with ECs. In addition, Cyp1b1-deficient PCs showed sustained activation of NF-κB, abnormalities in matrix secretion, and decreased production of vascular endothelial growth factor. These results confirm the important roles of PCs in development, maturation, and stabilization of blood vessels as well as in maintaining the cellular reductive state in PCs. Additional studies are needed to dissect the relative contributions of various PC functions to blood vessel formation, as indicated by the phenotypes identified in Cyp1b1^{-/-} knockout mice.

Analyzing acquired cancer therapy resistance in plasma

High-throughput DNA sequencing

is making it possible to do amazing things. An example is in-depth analysis of circulating tumor DNA that has been shed into the plasma. In a recent letter in *Nature*, Murtaza *et al* describe exome sequencing on a small number of cancer patients during chemotherapy that enabled the authors to detect several chemotherapy-resistance mutations. Because plasma contains representative DNA from a variety of tumor nodules, examining plasma DNA could provide a more global snapshot of resistance alleles during therapy, as compared with conventional tumor biopsies that suffer from sampling bias. Furthermore, analysis of plasma DNA requires only peripheral blood, which is easy to obtain. Although this is only a proof-of-principle study, it clearly proves the feasibility of this approach.

Nature 2013;497:108–112; doi:10.1038/nature12065

New reasons to avoid eating red meat Just when you thought you thoroughly understood cardiac risk factors associated with eating red meat, Koeth *et al*, as reported in an article in *Nature Medicine*, have identified a totally unexpected relationship between red meat consumption and risk of cardiovascular disease (CVD). They demonstrated that L-carnitine, which is found in abundance only in red meat, is metabolized

by certain gut bacteria to trimethylamine, which is rapidly converted to trimethylamine-N-oxide (TMAO). Mechanistically, TMAO contributes to CVD through inhibition of reverse cholesterol transport, thereby increasing plasma cholesterol and plaque formation. Because only certain kinds of bacteria can metabolize L-carnitine, these results suggest that modification of gut flora by antibiotics could prevent CVD due to ingestion of L-carnitine. Moreover, the use of L-carnitine as a common dietary supplement must now be evaluated in light of this study's findings.

Nature Medicine, published online 7 April 2013; doi:10.1038/nm.3145

Potential therapy for fragile X syndrome

Fragile X syndrome (FXS) is the most common monogenic cause of inherited intellectual disability and autism. Unstable expansion of CGG repeats in the 5' untranslated region of *FMR1* inhibit its expression and the protein it encodes, fragile X mental retardation protein (FMRP). Uncontrolled activity of group I metabotropic glutamate receptors has been implicated in the pathogenesis of FXS through uncontrolled synthesis of endocannabinoids, eliciting endocannabinoid system (ECS) receptor downregulation and long-term depression of the ECS. As recently described in a letter in *Nature Medicine*, this led Busquets-Garcia *et al* to hypothesize that inhibition of ECS receptors might be useful in treating FXS. They utilized a mouse model of FXS that recapitulates its salient features. The researchers found that inhibition of ECS signaling either directly or indirectly, through inhibition of downstream mTOR signaling, was effective in normalizing many FXS traits.

Nature Medicine, published online 31 March 2013; doi:10.1038/nm.3127

POT1 mutations in chronic lymphocytic leukemia

To gain insight into the genetic mutations that cause chronic lymphocytic leukemia (CLL), Ramsay *et al*, as reported in a recent letter in *Nature Genetics*, sequenced the exomes of matched tumor and normal samples from 127 patients with untreated CLL. In addition to mutations that had already been identified in CLL, such as mutations in *NOTCH1*, they identified mutations in *POT1*, a member of the family of shelterin proteins, which are critical regulators of telomere integrity. In CLL tumors with mutations in *POT1*, the incidence of telomere-containing chromosomal fusions was higher than in CLL tumors that did not have *POT1* mutations. Furthermore, patients with *POT1* mutations presented with more advanced disease at diagnosis and worse prognostic features than those without *POT1* mutations, suggesting that *POT1* mutations lead to tumor progression in CLL.

Nature Genetics 2013;45:526–530; doi:10.1038/ng.2584