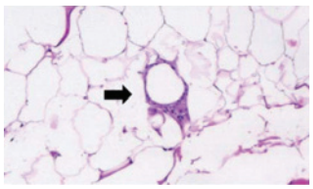
Cancer Research

Breaking Advances
Highlights from Recent Cancer Literature

Obesity, Inflammation, and Cancer



Obesity is strongly linked to increased incidence of several malignancies, including hormone receptor–positive breast cancer. Because excess adipose tissue secretes inflammatory mediators, obesity is also associated with a state of low-grade, chronic inflammation. Breast adipose tissue in overweight and obese women contains distinct infiltrates of macrophages that are a source of growth factors and inflammatory cytokines. A mechanistic explanation now links obesity-associated inflammation with hormone alterations in human breast tissue. Subbaramaiah and colleagues show how prostaglandin E_2, generated by the inflammatory enzyme cyclooxygenase (COX)-2, stimulates a cyclic AMP–PKA signal transduction pathway leading to increased levels of the estrogen-generating enzyme, aromatase and progesterone receptors in breast tissues from overweight and obese women. The in-breast inflammation index in these tissues, assessed by staining for macrophage infiltrates, was an even better correlate for increased inflammatory enzymes and hormone alterations than body mass index. There is a well-established role for COX-2 in cancer-related inflammation. Nonsteroidal inflammatory drugs (NSAID), such as aspirin, both inhibit COX-2 and have cancer-preventative actions, most notably in colorectal cancers, but also in breast cancer. The link between inflammation, obesity, and hormone activity suggests that NSAIDs may reduce estrogen levels and estrogen receptor–positive cancer. Taken together, this information suggests that dietary, behavioral, and pharmacologic strategies (e.g., combining low doses of NSAIDS and aromatase inhibitors) should be tested in overweight and obese women in combination to diminish inflammation, reduce cancer risk, and improve outcome for breast cancer survivors. *(Image from cited article courtesy of publisher.)*

Subbaramaiah K, Morris PG, Zhou XK, Morrow M, Du B, Giri D, et al. Increased levels of COX-2 and prostaglandin E_2 contribute to elevated aromatase expression in inflamed breast tissue of obese women. Cancer Discov 2012;2:356–65.

PIK3CA Mutations in Breast Cancer Detected in Plasma

Mutational analysis of targetable genes is increasingly used to guide therapeutic decisions. While single-gene or panel mutation screening can be applied clinically on tumor samples, this approach has clear limitations. Logistics become a concern if patient care and diagnostics are performed at different centers. In the case of metastatic cancer, it can be difficult to obtain tissue from a metastatic lesion, and mutational analysis of the primary lesion may not reflect the state of the metastatic tumor. Minimally invasive methods to detect mutations in tumors can help address these challenges. Tumors are known to shed nucleic acid into the blood, leading Higgins and colleagues to a method called BEAMing (beads, emulsification, amplification, and magnetics) to detect PIK3CA mutations in the plasma of patients with breast cancer. In BEAMing, individual DNA molecules are attached to magnetic beads in water–oil emulsions and then subjected to PCR amplification. Hybridization to fluorescent allele-specific probes for mutant or wild-type PIK3CA is used to determine mutational status, and flow cytometry is then applied to quantify levels of mutant DNA in the plasma. In this study, the authors retrospectively tested a cohort of tumors and temporally matched plasma samples for several hotspot mutations in PIK3CA and found 100% concordance. They then prospectively collected serum samples from patients with recurrent/metastatic disease, and interestingly found differences (in both directions) in approximately 25% of cases when compared with the primary tumor. Discordant results were observed when more than 3 years had passed between the primary diagnosis and time of blood collection, suggesting changes in tumor biology. These observations indicate that a targeted agent relevant to the current tumor burden should be chosen at the time of metastases, and the finding of temporal differences points to the importance of screening methods that can be used over time. The quantitative advantage of the BEAMing method potentially allows physicians to follow disease burden over time. The method is limited by the fact that it is hotspot based and therefore can be used only to identify mutations prespecified on the basis of incidence and/or relevance as a drug target. However, even with this caveat, the use of BEAMing and similar minimally invasive methods to screen for mutations in solid tumors will likely become more widespread as new targeted therapies and predictive markers are developed.

Higgins MJ, Jelovac D, Barnathan E, Blair B, Slater S, Powers P, et al. Detection of tumor PIK3CA status in metastatic breast cancer using peripheral blood. Clin Cancer Res; Published OnlineFirst March 15, 2012; doi:10.1158/1078-0432.CCR-11-2696.

Marginal Importance in Breast Cancer

For patients with ductal carcinoma *in situ* (DCIS) of the breast who receive breast-conserving surgery, negative surgical margins (absence of tumor at the resection edges of the specimen) are associated with reduced risk of recurrence in the same (ipsilateral) breast. Typically, the closest distance in the specimen between the surgical margin and the neoplastic cells is reported. However, little is known about the optimal amount of negative margin width with respect to prevention of ipsilateral breast tumor recurrence. In a meta-analysis, Wang and colleagues examined data from 7,564 patients with DCIS who received breast-conserving surgery. Patients were separated into 2 groups based on those who received surgery alone ($n = 3{,}098$) and those who received radiotherapy after surgery ($n = 4{,}466$). As expected, patients with positive tumor margins (both surgery-only and surgery plus radiotherapy groups) experienced a higher rate of ipsilateral tumor recurrence compared with those with negative margins. The authors then examined the margin width and tumor recurrence, finding that patients with a margin width greater than 10 mm had a significantly lower recurrence rate than patients with 2-mm margins. The results indicate that wide negative surgical margins should be attempted, where possible, to prevent tumor recurrence. In an accompanying editorial, Morrow and Katz note that margins greater than 10 mm were achieved in less than 10% of the patients in this cohort. They also discuss nonstandardized tissue processing methods and several possible confounding variables. Patients with larger margins were more likely to have been treated more recently than those with smaller margins, and rates of local recurrence have decreased in recent years for reasons not attributable to margin

width. Additionally, it is possible that the patients with wide resections might have been skewed to a more favorable risk category (e.g., lower grade tumor or higher rates of tamoxifen treatment) in such a way that the decreased recurrence rate would have been observed even without a wide resection. A study from the Eastern Cooperative Oncology Group did not support the finding of this meta-analysis. In addition, the risk of dying of breast cancer with a negative margin of any size is very low, indicating that the clinical benefit of a large margin would be very small. Additional observational studies, perhaps with standardized tissue processing protocols, are required to fully evaluate if a wider negative surgical margin in DCIS results in a clinical benefit.

Wang SY, Chu H, Shamliyan T, Jalal H, Kuntz KM, Kane RL, Virnig BA. Network meta-analysis of margin threshold for women with ductal carcinoma in situ. J Natl Cancer Inst 2012;104:507–16.

Morrow M, Katz SJ. Margins in ductal carcinoma in situ: Is bigger really better? J Natl Cancer Inst 2012;104:494–5.

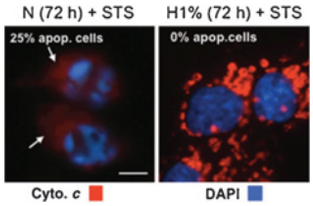
PI3K Suppresses BRAF-Driven Senescence in Melanoma

Although nevi (moles) represent benign melanocytic lesions that can be precursors to malignant melanoma, identification of the molecular and cellular events driving progression remains elusive. Oncogene-induced senescence (OIS) has been proposed to limit progression of benign to malignant melanomas. Vredeveld and colleagues show that deletion of the *PTEN* tumor suppressor or manipulation of the PI3K pathway (by ectopic expression of the catalytically active PI3 kinase) cooperate with a specific mutation of BRAF ($BRAF^{600E}$) to bypass cell cycle arrest and OIS in human fibroblasts, as well as human melanocyte cultures. The authors delve into the functional mechanism of bypassing OIS *in vivo* by showing that *PTEN* deletion cooperates with $BRAF^{600E}$ mutation, generating tumors in a mouse model for melanoma. The authors next analyzed human tissue sections harboring nevi in direct contiguity with melanomas. Identical mutations in BRAF and NRAS are present in benign and malignant melanocytes. This analysis also revealed a recurrent activation of the PI3K pathway in melanomas relative to contiguous nevi, suggesting that activation of PI3K might be a prognostic marker for tumor progression. Finally, employing human melanoma cell lines, the authors demonstrate that pharmacologic inhibition of PI3K and BRAF cooperate to produce cytotoxicity. Most interestingly, this combination is also efficacious for eliminating populations of melanoma cells resistant to BRAF inhibition. This article not only sheds light on important mechanisms involved in melanoma formation but also presents a therapeutic rationale for simultaneously targeting BRAF and PI3K in melanoma.

Vredeveld LCW, Possik PA, Smit MA, Meissl K, Michaloglou C, Horlings HM, et al. Abrogation of BRAFV600E-induced senescence by PI3K pathway activation contributes to melanomagenesis. Genes Dev 2012;26:1055–69.

Role for a Truncated Active Form of Voltage-Dependent Anion Channel 1 in Lung Cancer

The inability of chemotherapy to effectively induce apoptosis in tumor cells represents a significant impediment to developing effective cancer therapies. Moreover, the precise role of



mitochondrial alterations in hypoxia-driven chemotherapy resistance is poorly understood. Brahimi-Horn and colleagues demonstrate that some chemotherapy-resistant hypoxic tumor cells contain enlarged mitochondria with reorganized cristae, a result of modifications in fusion/fission, implicating an association and potential involvement of mitochondrial proteins with hypoxia-associated resistance to chemotherapy. A truncated, but active, version of voltage-dependent anion channel 1 (VDAC1), which plays a key role in the regulation of mitochondrial metabolism and apoptosis, contributed to increased cell survival in hypoxia via interaction with the antiapoptotic molecule, hexokinase II. Moreover, the truncated version of the VDAC1 (VDAC1-del) was detected in primary human lung adenocarcinomas, particularly at advanced stages. In these contexts, VDAC1-del appears to be a key molecule involved in chemotherapy resistance favoring tumor progression, and thus could be useful to develop strategies for disease monitoring and therapy in chemotherapy-resistant patients. *(Image from cited article courtesy of publisher.)*

Brahimi-Horn MC, Ben-Hail D, Ilie M, Gounon P, Rouleau M, Hofman V, et al. Expression of a truncated active form of VDAC1 in lung cancer associates with hypoxic cell survival and correlates with progression to chemotherapy resistance. Cancer Res 2012;72:2140–50.

Metabolic Pathways Controlling KRAS-Driven Pancreatic Cancer

Mechanisms by which different oncogenic pathways alter distinct nodes in cellular metabolism, and how these events converge on tumor formation remain poorly understood. In this study, Ying and colleagues establish a novel inducible $KRAS^{G12D}$-driven mouse model of pancreatic ductal adenocarcinoma that relies strictly on oncogenic KRAS expression for both tumor development and maintenance. Microarray profiling and metabolomics analyses of tumors and cells expressing oncogenic KRAS revealed enrichment in genes involved in glucose metabolism–in particular, genes within the hexosamine and ribose biosynthetic pathways. The authors further demonstrate that the metabolic response to oncogenic KRAS relied upon KRAS-driven increases in MYC expression, which led to transcriptional upregulation of hexosamine and pentose phosphate pathway genes. Importantly, knockdown of key regulated hexosamine and ribose biosynthesis pathway genes restrained the tumorigenicity of oncogenic KRAS-expressing cells, suggesting the dependence of KRAS-driven tumors on altered glucose utilization. This article adds to the growing list of studies that are uncovering oncogene-dependent alterations in cellular metabolism. More importantly, this study identifies novel targets that might be exploited for the treatment of KRAS-driven pancreatic ductal adenocarcinoma, a disease for which current treatments are woefully inadequate.

Ying H, Kimmelman AC, Lyssiotis CA, Hua S, Chu GC, Fletcher-Sananikone E, et al. Oncogenic Kras maintains pancreatic tumors through regulation of anabolic glucose metabolism. Cell 2012;149:656–70.

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.