

constitutes a seminal discovery that adds paternal inheritance as a new variable to be considered in the genetic susceptibility field.

Comment. With the publication of the first complete human genome draft in 2001, the identification of causative genes for human diseases was tremendously facilitated. This thorough knowledge of our DNA sequence and its common variation permitted us the ability to generate molecular tools, such as DNA microarrays, that can type a single DNA sample or thousands of them with whole-genome coverage. Genetic association studies that compare frequency of genetic variations or SNPs among disease cases and controls could be finally pangenomic and unbiased and, therefore, they could leave behind the subjective approach of selecting a few specific candidate genes or regions. GWAS have been quite successful in the last 4 years, being able to identify >500 highly significant associations of common genetic variants with >80 diseases and traits (Proc Natl Acad Sci U S A 2009;106:9362–9367; <http://www.genome.gov/gwastudies>), including 85 regions that have been conclusively associated in over a dozen different cancers (Carcinogenesis 2010;31:111–120).

Although the GWAS strategy has accomplished with success the identification of new genetic susceptibility variants for many common diseases, there are most likely some uncovered issues that could explain why some of the heritability in common diseases remain unidentified (Nature 2009;461:747–753). Besides the single-locus analysis strategy performed in most GWAS, future efforts in this field should also be focused among others in precise phenotyping, accurate functional effects, structural and rare variants, population heterogeneity, interactions between loci, and environmental modulation. Analyzing for all these parameters among others would probably increase the achievements obtained by GWAS.

The Kong et al article reports for the first time parent-of-origin-specific effects for genetic susceptibility variants identified by a GWAS strategy, adding a new layer of complexity to this approach that will help to explain a fraction of the missing heritability. Parental origin has been not analyzed because it requires information not readily available for most GWAS, but the authors were able to perform such an approach by having access to the Iceland population with careful genealogic record keeping traced back >10 generations.

Genomic imprinting is the a priori most likely phenomenon to lead to a parental-origin-specific disease association although other parent-of-origin effects have been documented, including parental-specific mutations. Genomic imprinting causes certain genes to be expressed in a parent-of-origin-specific manner. Imprinted genes are either expressed only from the allele inherited from the mother, or in other instances from the allele inherited from the father. Forms of genomic imprinting have been

demonstrated in insects, mammals, and flowering plants. Genomic imprinting is an epigenetic process that involves methylation and histone modifications to achieve monoallelic gene expression without altering the genetic sequence. These epigenetic marks are established in the germline and are maintained throughout all somatic cells of an organism, and there are available databases that catalogue sites in our genome with genomic imprinting (Nucleic Acids Res 2001;29:275–276); www.otago.ac.nz/IGC).

The authors focused on genetic variants previously associated with diseases that were located in imprinted regions of the human genome to test for the hypothetical parental effect, and they successfully identified such an effect in 5 of them. However, 2 of the analyzed variants did not show any parent-of-origin-specific effect, implying that this mechanism could not be generalized for all genetic susceptibility variants located in imprinted regions. Additionally, they also identified 1 additional genetic variant for T2D located in a region that they further characterize to be a new genomic imprinted area.

To conclude, Kong et al establish a new mechanism of action for genetic susceptibility variants previously unforeseen. Their new approach and further knowledge of new genomic imprinted regions in the human genome will most probably help to increase in the near future the heritability explained by these genomic variations identified through GWAS.

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TO BEAD OR NOT TO BEAD: IS TRANSARTERIAL CHEMOEMBOLIZATION WITH DOXORUBICIN-ELUTING BEADS A NEW STANDARD OF CARE IN HEPATOCELLULAR CARCINOMA?

Lammer J, Malagari K, Vogl T, et al. (Cardiovascular and Interventional Radiology, Medical University Vienna, Vienna, Austria). Prospective randomized study of doxorubicin-eluting-bead embolization in the treatment of hepatocellular carcinoma: results of the PRECISION V study. Cardiovasc Intervent Radiol 2010;33:41–52.

Reyes DK, Vossen JA, Kamel IR, et al. (Department of Radiology, Johns Hopkins University Hospital, Baltimore, Maryland). Single-center phase II trial of transarterial chemoembolization with drug-eluting beads for patients with unresectable hepatocellular carcinoma: initial experience in the United States. Cancer J 2009;15:526–532.

Transarterial chemoembolization (TACE) using an empirical mixture of lipiodol and cytotoxic agents followed by an embolic agent is accepted as standard therapy for intermediate stage hepatocellular carcinoma (HCC) worldwide. To further improve the selective delivery and maintenance of intra-arterial therapeutic agents doxorubicin-eluting beads (DEB) have been developed. Recently, the results of 2 independent phase II trials from Europe and the United States investigating the use of DEB-TACE have been published.

In the European multicenter trial, 212 patients with unresectable HCC, including those tumors unsuitable for percutaneous ablation, were randomized to receive either conventional TACE with lipiodol and doxorubicin emulsion followed by an embolic agent or DEB-TACE (*Cardiovasc Intervent Radiol* 2010;33:41–52). The per-protocol analysis consisted of 108 patients in the TACE and 93 patients in the DEB-TACE group of different Barcelona Clinic Liver Cancer (BCLC) stages. The 2 groups were well balanced and none of the patients had a BCLC stage C (advanced). Eastern Cooperative Oncology Group performance status (ECOG PS) was 0 (77%) or 1 (23%), and 83% of patients had a Child–Pugh stage A stage liver cirrhosis. The mean tumor size was 8.9 cm in both groups, and the majority of patients had binodular or multifocal disease. The primary end point was objective tumor response after 6 months based on the European Association for the Study of the Liver criteria ($\geq 30\%$ decrease of arterial tumor enhancement). According to the per-protocol analysis objective, response rates in the TACE and DEB-TACE groups did not significantly differ (44% vs 52%; $P = .11$). Nevertheless, patients with more advanced disease—defined as Child–Pugh stage B, ECOG PS 1, bilobular or recurrent disease—had a significantly greater objective response rate in the DEB-TACE group (35% vs 53%; $P = .038$). The incidence of adverse events was lower in the DEB-TACE group compared with the TACE group. Notably, liver toxicity was significantly lower in the DEB-TACE group (1 patient compared with 23 patients in the TACE group), and grade 3/4 bone marrow suppression occurred in only 1 patient in the DEB-TACE group, but in 6 patients in the TACE group.

Reyes et al reported a single-arm study of 20 patients, with unresectable HCC enrolled at The Johns Hopkins University Hospital between December 2005 and December 2007 (*Cancer J* 2009;15:526–532). BCLC stage A was present in 6 patients, BCLC stage B in 2, and BCLC stage C in 12. ECOG PS was 0 or 1 in 19 of the 20 patients, and 15 patients had a Child–Pugh stage A stage liver cirrhosis. The mean tumor size was 6.9 cm (range, 1.9–16.2), and 16 patients had a solitary nodule with or without satellites. Two patients had 2 lesions and the remaining 2 patients had multifocal lesions. A portal vein thrombosis was present in 4 patients. Two DEB-TACE cycles were delivered (range, 1–3), and the median follow-up time

was 14.5 months. DEB-TACE was well tolerated with modest toxicity in most patients. One patient developed grade 3 leukocytopenia and another patient developed pancreatitis, most likely owing to backflow of microspheres. Two patients died within 30 days after DEB-TACE, but both cases were judged as unrelated to the procedure. In 1 patient with a subcapsular HCC, tumor rupture within 24 hours after DEB-TACE occurred. A partial response—based on the modified RECIST criteria—was achieved in 2 patients, and the remaining 18 patients had a stable disease measured by magnetic resonance imaging 1 month after initial DEB-TACE. At 6 months, the disease control rate (objective response plus stable disease) was 95%. Additionally, relevant changes in contrast enhancement as a surrogate marker for tumor necrosis were reported in the majority of patients. In 6 patients, a complete loss of arterial tumor enhancement was documented, and in 6 additional patients a decrease of $>30\%$ was reported. According to the European Association for the Study of the Liver criteria, this translates into an objective response rate of 60%. In 4 patients who became resectable after DEB-TACE, histopathologic examination of the specimen revealed a close correlation between the degree of tumor necrosis (80%–95%) and decrease contrast enhancement. The median progression-free and overall survivals were 13 and 26 months, respectively; the 1- and 2-year survival rates were 65% and 55%, respectively.

Comment. The incidence of HCC, among the most severe complications of liver cirrhosis, is rising worldwide. Currently, the annual incidence is estimated at $>600,000$ new cases and the overall prognosis is poor. In Western countries, treatment of patients with HCC is stratified according to the BCLC algorithm, which differs between very early (0), early (A), intermediate (B), advanced (C), and terminal (D) stage (*Semin Liver Dis* 1999;19:329–338). Although percutaneous tumor ablation, liver resection, and liver transplantation are potential curative treatment options for very early and early stage HCC, expansion of median survival can be achieved for intermediate stage HCC by TACE and in advanced stage by systemic treatment with sorafenib (*N Engl J Med* 2008; 359:378–390; *Lancet* 2009;373:614–616).

A new development for intra-arterial therapy is drug-eluting microspheres, such as DEB. These loaded beads, 100–900 μm in diameter, have been designed to allow a higher and more sustained release of cytotoxic drugs directly into the tumor and to reduce systemic side effects. In a previous pilot study in patients with HCC, Varela et al demonstrated promising efficacy and lower systemic doxorubicin levels compared with conventional doxorubicin-based TACE (*J Hepatol* 2007;46: 474–481). These findings were now confirmed by the randomized trial reported by Lammer et al. They showed lower rates of systemic toxicity in patients treated with

DEB-TACE compared with conventional TACE. However, the PRECISION V study is formally a negative study, because patients in the conventional TACE group had a higher response than expected (44%; expected, 35%). Consequently, this study was underpowered to detect a significant difference for the primary study end point. However, patients with advanced tumor stage—defined as Child–Pugh stage B, ECOG PS 1, bilobular or recurrent disease—had a significantly greater response rate when treated with DEB-TACE. The finding that patients with advanced HCC benefit from DEB-TACE is further supported by the monocentric pilot study from Reyes et al. In their study, 60% patients had a BCLC stage C, a tumor stage where systemic treatment with sorafenib is an alternative. Of note, the safety profile in these patients was acceptable.

A major criticism of the study by Lammer et al is the lack of 2 essential end points of clinical trials in oncology: progression-free and overall survival. Because these end points are the hardest criteria for superiority in clinical oncology, the impact of a higher response on outcome remains uncertain. In the study by Reyes et al, these end points were addressed. However, because of the small sample size and missing randomization, the question of whether DEB-TACE is superior to conventional TACE cannot be answered, so far. Will further randomized studies comparing DEB-TACE with conventional TACE be conducted? Probably not, because large studies combining DEB-TACE with sorafenib in randomized, controlled trials are currently enrolling patients. These data will become available in the next 2 years. Should we offer DEB-TACE as a new standard in the meantime? Not to all patients with intermediate stage HCC. Based on the data provided by Lammer et al and Reyes et al, patients with more advanced disease may benefit most from DEB-TACE. Therefore, DEB-TACE might expand the indications of intra-arterial therapy for more fragile patients with more advanced disease. DEB-TACE could therefore turn out as a cost-effective alternative to radioembolization with yttrium-90 microspheres, another promising new technique with high antitumoral effects and a good toxicity profile (*Gastroenterology* 2010;138:52–64).

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HIPPO TUMOR SUPPRESSOR PATHWAY: NOVEL IMPLICATIONS FOR THE TREATMENT OF HEPATOCELLULAR CARCINOMA

Zhou D, Conrad C, Xia F, et al. (Department of Molecular Biology, Massachusetts General Hospital, Boston,

Massachusetts). Mst1 and Mst2 maintain quiescence and suppress hepatocellular carcinoma development through inactivation of the Yap1 oncogene. *Cancer Cell* 2009;16:425–438.

Hepatocellular carcinoma (HCC) remains the main cause of death in cirrhotic patients and the third most common cause of cancer related death worldwide (*CA Cancer J Clin* 2005;55:74–108). Curative treatment options are restricted to <30–40% of patients due to disease progression at diagnosis (*Lancet* 2003;362:1907–1917), demanding better surveillance programs for early tumor detection and the development of both chemopreventive treatments and novel treatment strategies. A multikinase inhibitor, sorafenib greatly succeeded in the treatment of advanced HCC and became the only therapy approved by the US Food and Drug Administration, clearly demonstrating that molecular targeted therapy is a realistic treatment option in this cancer (*N Engl J Med* 2008;4:378–390). This success has facilitated many pre-clinical and clinical trials investigating novel small molecules and monoclonal antibodies for the treatment of HCC (*Hepatology* 2008;48:1312–1327). In the future, more precise and comprehensive characterization of molecular classes, genetic and epigenetic changes, and altered cell signaling pathways should be taken into account to choose the right treatment scheme for each patient, leading to a much more personalized medicine in HCC (*Semin Liver Dis* 2010;30:35–51).

Wnt- β -catenin, p53, IGF, Ras-MAPKK, PI3/Akt/mTor, and c-Met are well-studied signaling pathways involved in the development and progression of HCC. Recently, genetic and biological studies confirmed the importance of the novel Hippo tumor suppressor pathway in regulating cell proliferation, apoptosis, organ size, and tumorigenesis in mammals (*Cancer Cell* 2008;13:188–192). This relatively new pathway was first described in *Drosophila* in 1995 by identifying the tumor suppressor Warts, followed by the establishment of several other core Hippo pathway components (as shown in Figure 1; *Curr Opin Cell Biol* 2008;20:638–646). In mammalian cells, NF2, FRMD6, and FAT4 are upstream regulators of the MST1/2 kinases that physiologically form a complex with WW45 upon activation to then phosphorylate and activate the downstream kinases LATS1/2. This core complex together with MOB1 phosphorylates and inhibits the transcription coactivators YAP1 and TAZ by causing their cytoplasmic translocation (*Nat Rev Mol Cell Biol* 2007;8:613–621; *Nat Rev Cancer* 2007;7:182–191).

Many of these Hippo pathway members have been implicated in the genesis of cancer. NF2 is mutated or silenced in familial (neurofibromatosis type 2) and sporadic human tumors, MOB1 deletion has been shown in human melanoma and mouse mammary gland carcinoma. LATS1 deficient mice develop ovarian tumors and soft tissue sarcomas and both LATS1 and 2, have been