Emerging Roles of DMP1 in Lung Cancer
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Abstract
The Ras-activated transcription factor DMP1 can stimulate Arf transcription to promote p53-dependent cell arrest. One recent study deepens the pathophysiologic significance of this pathway in cancer, first, by identifying DMP1 losses in human lung cancers that lack ARF/p53 mutations, and second, by demonstrating that Dmp1 deletions in the mouse are sufficient to promote K-ras-mediated lung tumorigenesis via mechanisms consistent with a disruption of Arf/p53 suppressor function. These findings prompt further investigations of the prognostic value of DMP1 alterations in human cancers and the oncogenic events that can cooperate with DMP1 inactivation to drive tumorigenesis.

Genetic Alterations in Human Lung Cancer
Lung cancer is the leading cause of cancer deaths in the United States and worldwide. Despite the recent advances of surgical and chemo/radiation therapies, the disease is rarely curable and the prognosis is very poor, with an overall 5-year survival rate of only 15% (1). The unfortunate outcome of lung cancer is mainly explained by the difficulty of early detection and anatomic localization of the tumors (1). Lung cancer can be categorized into two major histopathologic groups: non–small cell lung cancer (NSCLC) and small cell lung cancer (SCLC; ref. 2). Approximately 80% of human lung cancers are NSCLC, and they are subcategorized into adenocarcinomas, squamous cell, and large cell carcinomas. SCLC and NSCLC show major differences in histopathologic characteristics that can be explained by the distinct patterns of genetic alterations found in both tumor classes (3). For instance, the K-Ras gene is mutated in 20% to 30% of NSCLC, whereas its mutation is rare in SCLC; Rb inactivation is found in ~90% of SCLC, whereas p16INK4A is inactivated by deletion and/or promoter hypermethylation in ~50% of NSCLC (3). p14ARF (p19ARF in mice), an alternative reading frame gene product generated from the INK4a/ARF locus, is more frequently inactivated in SCLC (~65%) than in NSCLC (~20%), suggesting a distinctive role for p14ARF in human lung cancer suppression (3-5).

Among the dozens of murine models of human lung cancer, the K-rasLA/− (K-rasLA1/−, K-rasLA2/−) mouse model, which we use to study NSCLC, is one of the most sophisticated (3, 6). In this transgenic model, the mutant K-ras gene is regulated by its own promoter and is activated only during spontaneous recombination events within the whole animal (6). K-rasLA1/− and K-rasLA2/− models differ in that K-rasLA2 has two mutant copies of exon 1, whereas in K-rasLA1, only the upstream copy of exon 1 is mutant (6). K-rasLA1-mediated lung carcinogenesis was strikingly accelerated in mice of both p53+/− and p53−/− backgrounds, reflecting the frequent alteration of p53 in K-rasLA lung tumors and human lung cancers (3, 6, 7). On the other hand, the Ink4a/Arf locus is not frequently altered in these K-rasLA lung cancer models, and the results of crossing K-rasLA transgenic mice with Arf and/or Ink4a-null mice had not been previously reported.

Signaling Pathways Involving Dmp1
Cyclin D binding myb-like protein-1 (Dmp1; also called Dmtf1, cyclin D binding myb-like transcription factor 1) was originally isolated in a yeast two-hybrid screen of a murine T-lymphocyte library with cyclin D2 as bait (8). Dmp1 binds to nonameric (ERK)-ERK-Jun pathway, and the induction of Dmp1-null mice are prone to spontaneous tumor development, which was dramatically accelerated when neonatal animals were treated with ionizing radiation or dimethylbenzanthracene that causes Ras mutation (11, 12). Lung adenomas/adenocarcinomas were the most common tumors found in Dmp1-deficient mice.

Eμ-Myc transgenic mouse is a model of human Burkitt-type B-cell tumors (13). More than half of the lymphomas arising in Eμ-Myc mice have p53 mutations or biallelic Arf deletions (~25%), whereas others lacking overt Arf or p53 mutations overexpress Mdm2 (13). The survival of Eμ-Myc mice was significantly shortened in both Dmp1+/− and Dmp1−/− mice (12). The retention and expression of the wild-type Dmp1 allele in Eμ-Myc tumors arising in Dmp1+/− mice suggested that Dmp1 is haplo-insufficient for tumor suppression (12, 14). Moreover, the low frequency of Arf deletion and p53 mutation in tumors from Dmp1-knockout mice indicated that Dmp1 is a physiologic regulator of the Arf-p53 pathway (12, 14).

Information about the signaling cascades that regulate Dmp1 has been accumulating. The Dmp1 promoter is activated by the oncogenic Ras-Raf-MAP/extracellular signal-regulated kinase (ERK)-ERK-Jun pathway, and the induction of Arf by Ras is Dmp1-dependent (15). On the other hand, our recent study shows that both the activity of the Dmp1 promoter and Dmp1 mRNA are repressed by overexpression of E2F1, 2, 3a, 3b, and 4 and by serum stimulation (16). Repression of the Dmp1 promoter by serum was dependent on E2Fs because overexpression E2F-DB, a deletion mutant of E2F1 that lacks the transactivation domain, relieved the repression (16). Whereas both Dmp1 and Arf mouse promoters are repressed by anthracyclin anticancer drugs and by UV-C, we found that this repression is mediated by direct binding of the nuclear factor-eB subunit, p65, to the Dmp1 promoter (17).
Roles of Dmp1 in K-ras Models of Lung Cancer

To investigate the cooperative effects of Dmp1 loss and oncogenic K-ras activation in vivo, compound mice were created by crossing Dmp1-deficient mice with K-rasLA2/+ or K-rasLA1/+ mice (6, 7). K-rasLA lung cancer was significantly accelerated in both Dmp1+/− and Dmp1−/− mice, with no differences between groups of Dmp1+/− and Dmp1−/− (7). Lung tumors from Dmp1+/−, K-rasLA2/+, and K-rasLA−/− mice retained the wild-type Dmp1 allele when examined by genomic DNA PCR, and half of them expressed Dmp1 mRNA (and protein) at levels that were two to four times higher than in nontransgenic Dmp1+/− lungs (7). However, the Dmp1 mRNA expression was at the same or lower level in the other half of Dmp1−/− lung tumors, suggesting that the signaling pathway between Ras and Dmp1 had been disconnected during carcinogenesis (7, 15). Our data showed a typical case of haploid insufficiency of Dmp1 in suppressing K-ras−induced lung tumors.

Approximately half of the lung tumors from Dmp1+/− or Dmp1−/− K-rasLA mice were adenocarcinomas with various degrees of differentiation and many showed signs of intravascular or intrabronchial invasion (7). In wild-type K-rasLA lung tumors, mutant p53 was expressed in ~40% of the samples, the frequency of which was considerably decreased (<10%) in tumors from Dmp1+/− or Dmp1−/− mice (7). Mdm2 overexpression or biallelic deletion of the p53, Arf, or Ink4a genes was not found in any of the lung tumors examined (7). None of the Ink4a/Arf regulators (Bmi1, Twist, Tbx2/3, and Pokemon) were overexpressed in K-rasLA lung carcinomas (7). Approximately 40% of lung tumors from Dmp1 wild-type K-rasLA mice showed a single allelic or a mixture of single allelic and biallelic deletions of the Dmp1 gene, which was not found in those with mutant p53 (7). The Dmp1 gene was not deleted in any of the lung tumor DNAs isolated from p53+/−, K-rasLA− or p53−/−, K-rasLA mice, showing mutually exclusive inactivation of Dmp1 and p53 in K-rasLA−mediated lung cancer (7).

Of note, the deletion of the Dmp1 locus was very selective in K-rasLA lung tumors because the grm3 and abcb1 genes located within 0.5 Mb of the Dmp1 locus were rarely affected (7). Collectively, our recent study showed that when lung carcinomas

Figure 1. Genomic structure of the human hDMP1 locus and the genomic regions deleted in NSCLC samples. There are only two genes at the hDMP1 locus between markers #69164 and #251945 (hDMP1 and MGC4175), and this locus was selectively deleted in 15 of 19 NSCLC samples (7). Most hDMP1 LOH(+) NSCLC samples are only weakly positive to negative for hDMP1 nuclear staining (bottom middle), whereas significant levels of the protein are detectable in lung cancers without LOH for hDMP1 (bottom left). The latter group often shows LOH for INK4a/ARF or that of p53, K-Ras mutations collaborate with DMP1 loss in lung carcinogenesis, reflecting the Dmp1 promoter activation by oncogenic Ras, but there are likely other oncogenic events that occur simultaneously with DMP1 inactivation. The pie charts show the relative frequency of the Dmp1 (hDMP1), Ink4a/Arf, and p53 involvement in human NSCLC and K-rasLA lung carcinomas. GRM3, glutamate receptor 3; KIAA1324L, KIAA1324 like; MGC4175, Mammalian Gene Collection 4175; CROT, carnitine O-octanoyltransferase; ABCB4, ATP-binding cassette, subfamily B (MDR/TAP), member 4; ABCB1, ATP-binding cassette, subfamily B (MDR/TAP), member 1. Figure modified with permission from Elsevier. Mallakin et al. Cancer Cell 2007;12:381–94(7).
arise from wild-type K-ras<sup>L4</sup> mice, the cells undergo either p53 mutation or Dmp1 deletion to inactivate the p53 pathway.

**hDMP1 and Human Lung Cancer**

The hDMP1 gene is located on human chromosome 7q21, a locus that is often deleted in therapy-induced acute leukemias, myelodysplastic syndromes, and some solid tumors (18, 19). Although Dmp1-deficient mice develop a variety of epithelial tumors (12), whether the human DMP1 gene (hDMP1) is involved in human carcinoma had never been investigated. We therefore extracted genomic DNA from >50 NSCLC samples and studied gender deletion by loss of heterozygosity (LOH) assays for hDMP1 (7). The hDMP1 locus was deleted in ~35% of human NSCLC as studied by two different sets of LOH primers (7). Detailed mapping of the genomic locus on human chromosome 7q21 deleted in human NSCLC showed that the genomic region deleted in NSCLC was confined to the hDMP1/MGC4175 locus in ~80% of hDMP1 LOH(+) cases (Fig. 1; ref. 7). Hypermethylation of the hDMP1 promoter was very rare in human NSCLC and none of the randomly chosen seven samples showed mutations for the hDMP1 gene, consistent with hDMP1 as a haploinsufficient tumor suppressor. We could not detect any lung cancerspecific overexpression of the hDMP1β isoform, which has a dominant-negative effect on hDMP1α (7, 20). Thus, hemizygous gene deletion is the major mechanism of hDMP1 inactivation in NSCLC (7).

Approximately 35% of our human NSCLC samples showed LOH (or biallelic deletion) for INK4a/ARF (7). In contrast to hDMP1, promoter hypermethylation was found in 7% for p14<sup>ARF</sup> and 50% for p16<sup>INK4a</sup>, consistent with previous reports from other groups (3). Most cases of the p16<sup>INK4a</sup> promoter hypermethylation were observed simultaneously with LOH of the locus (7). Some samples showed homozygous deletion of exon 1′ for p14<sup>ARF</sup>, suggesting that these two genes behaved as classic tumor suppressors in human NSCLC. Interestingly, ~90% of the NSCLC samples showed mutually exclusive inactivation of the hDMP1 and the INK4a/ARF loci (7). LOH of p53 was found in ~40% of our NSCLC samples, and again, LOH of hDMP1 and that of p53 tended not to overlap (7). On the other hand, inactivation of the INK4a/ARF locus and the p53 locus occurred more frequently together rather than exclusively (7), consistent with the previous study that showed coexistence of INK4a/ARF inactivation and p53 mutations in human NSCLC (21). This overlap can be explained by the fact that p16<sup>INK4a</sup> is more frequently involved than p14<sup>ARF</sup> in human NSCLC, and ARF has both p53-dependent and p53-independent functions (3, 5, 7, 21). In summary, our data showed that LOH of the hDMP1 gene was found in ~35% of human NSCLC, especially those that retain a wild-type INK4a/ARF and/or p53 locus. Of note, ~15% of hDMP1 LOH occurred simultaneously with K-Ras mutation, suggesting that our compound mouse models mimic human NSCLC from the viewpoint of synergism between Dmp1-loss and K-ras mutation in lung carcinogenesis.

To investigate the consequence of hDMP1 deletion in human NSCLC samples, expression of the hDMP1 protein was studied by immunohistochemistry. Strong nuclear staining (grade 3+ to 2−) was obtained in eight of eight hDMP1 LOH(−) lung cancer samples, whereas the staining was very weak (grade 1/+−) or negative (grade 0) in seven of nine hDMP1 LOH(+) lung cancer samples (7). Thus, the immunohistochemistry results showed reduced expression of the hDMP1 protein in LOH(+) lung cancer cells.

**Does hDMP1 Loss Define a New Category of Human Lung Cancer?**

Our study on human NSCLC samples clearly indicates that LOH of the hDMP1 gene and of the INK4a/ARF locus occurred in mutually exclusive fashion in >90% of cases, although some tumor samples showed inactivation of both hDMP1 and INK4a/ARF loci (7). Likewise, LOH of hDMP1 occurred much less frequently than expected in lung tumors that showed LOH for p53. Interestingly, one of the four human NSCLC cell lines (H460) showed hemizygous deletion of hDMP1, where both ARF and p53 are wild-type and K-Ras is mutated (7). Activated Dmp1ER efficiently induced p14<sup>ARF</sup> and inhibited the growth of H460 cells, whereas other lung cancer cell lines with deletion of ARF or p53 were resistant to growth arrest by Dmp1 overexpression (7). Consistent with the human data, the Dmp1 gene was deleted only in K-ras<sup>L4</sup> lung tumors with wild-type p53 but not in any of the lung tumors from p53−/− or p53<sup>−/−</sup>, K-ras<sup>L4</sup> mice (7). Collectively, the DMP1 gene is frequently deleted in lung carcinomas where the INK4a/ARF locus and/or the p53 locus remain wild-type. Thus, lung cancers with hDMP1 deletion might define a new disease category with better response to chemo/radiotherapy and longer survival of patients.

**Implications and Future Directions**

Out study shows that DMP1 is principally involved in human and murine pulmonary carcinogenesis. Future studies should focus on determining the prognostic values of hDMP1 deletion (or low hDMP1 expression in immunohistochemistry) in human NSCLC. SCLC and other human cancers should also be studied for hDMP1 deletion, mutations, and splicing alterations. Oncogenic events other than K-Ras mutation that collaborate with hDMP1 deletion should be investigated. We found that deletion of Dmp1 and mutation of p53 are mutually exclusive in K-ras<sup>L4</sup>–mediated lung carcinomas. Because p19<sup>Arf</sup> is not frequently involved in this particular lung cancer model, Dmp1 might regulate the p53 activity by yet unknown mechanisms in epithelial tissues, which should be investigated in the future. Dmp1 showed haploid insufficiency in K-ras<sup>L4</sup> murine lung tumors, and our data with lung cancer patients’ samples are compatible with haploid insufficiency of hDMP1 in NSCLC. Because hDMP1 LOH (+) lung cancer cells retain one allele of the hDMP1 locus, this gene might be a promising target for future anticancer drug screenings.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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