Published in final edited form as:
*Cancer Res.* 2008 June 15; 68(12): 4487–4490. doi:10.1158/0008-5472.CAN-07-6791.

# 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 pathophysiological 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*-induced 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.

### Keywords

Dmp1; K-ras; $p19^{Arf}$; $p14^{ARF}$; $p16^{Ink4a}$; p53; non-small-cell lung cancer (NSCLC); loss of heterozygosity; deletion; promoter hypermethylation; haploid insufficiency

## Genetic alterations in human lung cancer

Lung cancer is the leading cause of cancer deaths in the US 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 anatomical localization of the tumors (1). Lung cancer can be categorized into two major histopathological groups: non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC) (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 20-30 % of NSCLC, while its mutation is rare in SCLC; *Rb* inactivation is found in ~90 % of SCLC, while $p16^{INK4a}$ is inactivated by deletion and/or promoter hypermethylation in ~50 % of NSCLC (3). $p14^{ARF}$ ($p19^{Arf}$ 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 $p14^{ARF}$ in human lung cancer suppression (3-5).

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Among the dozens of murine models of human lung cancer, the *K-ras*$^{LA/+}$ (*K-ras*$^{LA1/+}$, *K-ras*$^{LA2/+}$) 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-ras*$^{LA1/+}$ and *K-ras*$^{LA2/+}$ models differ in that *K-ras*$^{LA2}$ has two mutant copies of exon 1, whereas in *K-ras*$^{LA1}$, only the upstream copy of exon 1 is mutant (6). *K-ras*$^{LA}$-mediated lung carcinogenesis was strikingly accelerated in mice of both *p53*$^{+/-}$ and *p53*$^{-/-}$ backgrounds, reflecting the frequent alteration of *p53* in *K-ras*$^{LA}$ lung tumors and human lung cancers (3, 6, 7). On the other hand, the *Ink4a/Arf* locus is not frequently altered in these *K-ras*$^{LA}$ lung cancer models, and the results of crossing *K-ras*$^{LA}$ transgenic mice with *Arf* and/or *Ink4a*-null mice had not been reported.

## Signaling pathways involving Dmp1

Dmp1 (cyclin D binding myb-like protein-1; 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 CCCG(G/T)ATG(T/C) DNA consensus sequences present on the *Arf* promoter (9, 10). Dmp1 directly binds to the *Arf* promoter to activate its expression, thereby inducing p53-dependent cell cycle arrest (10). *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 physiological 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-MEK-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 since 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 anti-cancer drugs and by UV-C, we found that this repression is mediated by direct binding of the NF-κB 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-ras*$^{LA2/+}$ or *K-ras*$^{LA1/+}$ mice (6, 7). *K-ras*$^{LA}$ 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-ras*$^{LA1/+}$ and *Dmp1*$^{+/-}$; *K-ras*$^{LA2/+}$ 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 2 to 4 times higher than in non-transgenic *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 demonstrated a typical case of haploid insufficiency of Dmp1 in suppressing *K-ras*-induced lung tumors.

Approximately half of the lung tumors from $Dmp1^{+/-}$ or $Dmp1^{-/-}$; $K\text{-}ras^{LA}$ mice were adenocarcinomas with various degrees of differentiation and many showed signs of intravascular or intrabronchial invasion (7). In wild-type $K\text{-}ras^{LA}$ 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* modulators (Bmi1, Twist, Tbx2/3, and Pokemon) were overexpressed in $K\text{-}ras^{LA}$ lung carcinomas (7). Approximately 40 % of lung tumors from *Dmp1* wild-type $K\text{-}ras^{LA}$ 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 $p53^{+/-}$; $K\text{-}ras^{LA}$ or $p53^{-/-}$; $K\text{-}ras^{LA}$ mice, showing mutually exclusive inactivation of *Dmp1* and *p53* in $K\text{-}ras^{LA}$-mediated lung cancer (7). Of note, the deletion of the *Dmp1* locus was very selective in $K\text{-}ras^{LA}$ lung tumors, since the *gram3* 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 arise from wild-type $K\text{-}ras^{LA}$ mice, the cells undergo either *p53* mutation or *Dmp1* deletion to inactivate the p53 pathway.

## h*DMP1* and human lung cancer

The h*DMP1* 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 (h*DMP1*) is involved in human carcinoma had never been investigated. We therefore extracted genomic DNA from more than 50 NSCLC samples and studied gene deletion by loss of heterozygosity (LOH) assays for h*DMP1* (7). The h*DMP1* 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 h*DMP1*/*MGC4175* locus in ~80 % of h*DMP1* LOH(+) cases (7). Hypermethylation of the h*DMP1* promoter was very rare in human NSCLC and none of the randomly chosen seven samples showed mutations for the h*DMP1* gene, consistent with h*DMP1* as a haplo-insufficient tumor suppressor. We could not detect any lung cancer-specific overexpression of the h*DMP1β* isoform, which has a dominant-negative effect on h*DMP1α* (7, 20). Thus, hemizygous gene deletion is the major mechanism of h*DMP1* inactivation in NSCLC (7).

Approximately 35 % of our human NSCLC samples showed LOH (or biallelic deletion) for *INK4a/ARF* (7). In contrast to h*DMP1*, promoter hypermethylation was found in 7 % for $p14^{ARF}$ and 50 % for $p16^{INK4a}$, consistent with previous reports from other groups (3). Most cases of the $p16^{INK4a}$ promoter hypermethylation were observed simultaneously with LOH of the locus (7). Some samples showed homozygous deletion of exon 1β for $p14^{ARF}$, suggesting that these two genes behaved as classical tumor suppressors in human NSCLC. Interestingly, ~90 % of the NSCLC samples showed mutually exclusive inactivation of the h*DMP1* and the *INK4a/ARF* loci (7). LOH of *p53* was found in ~40% of our NSCLC samples, and again, LOH of h*DMP1* 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 $p16^{INK4a}$ is more frequently involved than $p14^{ARF}$ in human NSCLC and ARF has both p53-dependent and -independent functions (3, 5, 7, 21). In summary, our data showed that LOH of the h*DMP1* 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 h*DMP1* 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 h*DMP1* deletion in human NSCLC samples, expression of the *h*DMP1 protein was studied by immunohistochemistry. Strong nuclear staining (grade 3++ to 2+) was obtained in 8 of 8 h*DMP1* LOH(−) lung cancer samples, while the staining was very weak (grade 1+/−) or negative (grade 0) in 7 of 9 h*DMP1* LOH(+) lung cancer samples (7). Thus, the immunohistochemistry results demonstrated reduced expression of the *h*DMP1 protein in LOH(+) lung cancer cells.

## Does h*DMP1* loss define a new category of human lung cancer?

Our study on human NSCLC samples clearly indicates that LOH of the h*DMP1* gene and of the *INK4a/ARF* locus occurred in mutually exclusive fashion in >90 % of cases, although some tumor samples showed inactivation of both h*DMP1* and *INK4a/ARF* loci (7). Likewise, LOH of h*DMP1* 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 h*DMP1*, where both *ARF* and *p53* are wild-type and *K-Ras* is mutated (7). Activated Dmp1:ER efficiently induced $p14^{ARF}$ and inhibited the growth of H460 cells, while 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 $K\text{-}ras^{LA}$ lung tumors with wild-type *p53* but not in any of the lung tumors from $p53^{+/-}$ or $p53^{-/-}; K\text{-}ras^{LA}$ 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 h*DMP1* deletion might define a new disease category with better response to chemo/radiotherapy and longer survival of patients.

## Implications and Future Directions

Our study shows that *DMP1* is principally involved in human and murine pulmonary carcinogenesis. Future studies should focus on determining the prognostic values of h*DMP1* deletion (or low *h*DMP1 expression in immunohistochemistry) in human NSCLC. SCLC and other human cancers should also be studied for h*DMP1* deletion, mutations, and splicing alterations. Oncogenic events other than *K-Ras* mutation that collaborate with h*DMP1* deletion should be investigated. We found that deletion of *Dmp1* and mutation of *p53* are mutually exclusive in $K\text{-}ras^{LA}$-mediated lung carcinomas. Since $p19^{Arf}$ 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 $K\text{-}ras^{LA}$ murine lung tumors, and our data with lung cancer patients' samples are compatible with haploid insufficiency of h*DMP1* in NSCLC. Since h*DMP1* LOH (+) lung cancer cells retain one allele of the h*DMP1* locus, this gene might be a promising target for future anti-cancer drug screenings.

## Acknowledgments

We would like to thank Drs. Charles Sherr, Martine Roussel, John Cleveland, Martin McMahon, Ali Mallakin, Lauren Matise, and Mark Willingham for their collaborative work on Dmp1 projects. We are grateful to Drs. Bruce Torbett and Mario Tschan for sharing unpublished data. We thank Ms. Karen Klein for editing. K. Inoue is supported by the NIH/NCI 5R01CA106314, the American Cancer Society RSG-07-207-01-MGO, and the Wake

Forest University Golfers against Cancer Grant P30CA12197GAC. D. Frazier is supported by the Ruth L. Kirschstein National Research Service Award Institutional Research Training Grant (5T32CA079448, F. Torti) from NIH.

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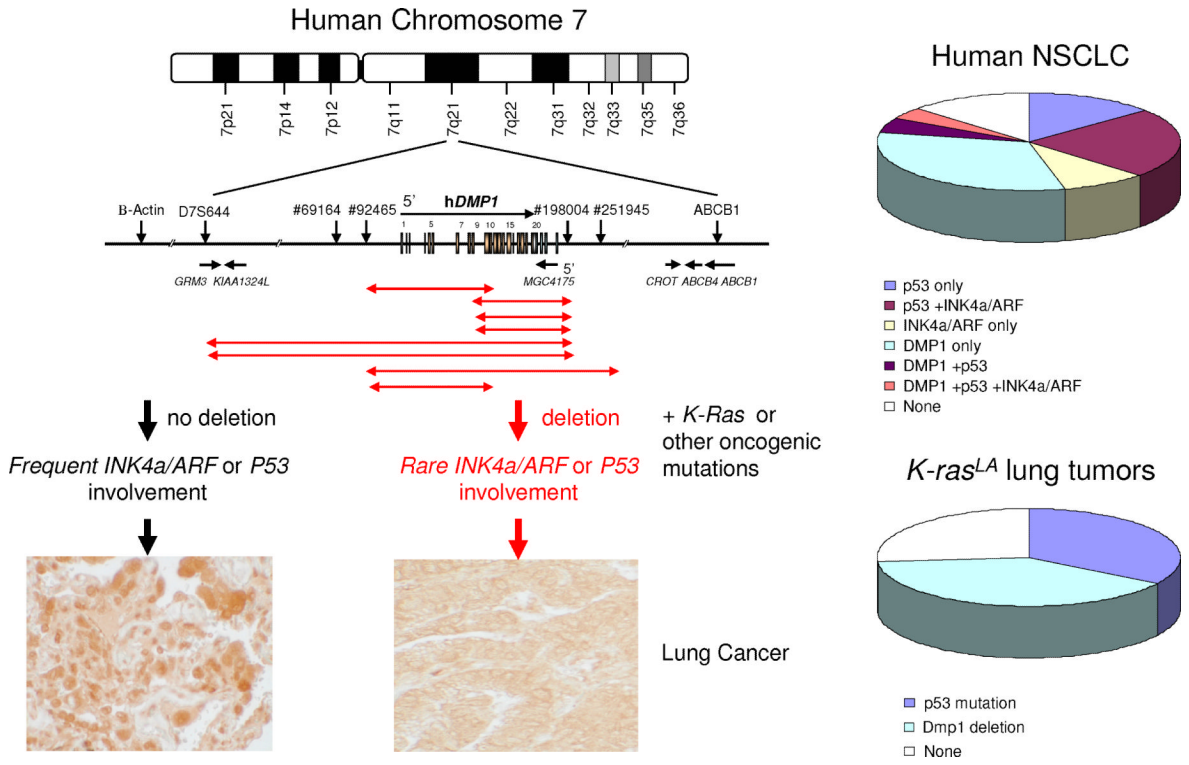


**Figure 1.**

Genomic structure of the human h*DMP1* locus and the genomic regions deleted in non-small cell lung cancer samples. There are only two genes at the h*DMP1* locus between markers #69164 and #251945 (h*DMP1* and *MGC4175*), and this locus was selectively deleted in 15 of 19 non-small-cell lung cancer (NSCLC) samples (7). Most h*DMP1* LOH(+) NSCLC samples are only weakly positive to negative for *h*DMP1 nuclear staining (bottom middle), whereas significant levels of the protein are detectable in lung cancers without LOH for h*DMP1* (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* (h*DMP1*), *Ink4a/Arf*, and *p53* involvement in human NSCLC and *K-ras*$^{LA}$ lung carcinomas. Abbreviations; GRM3: glutamate receptor 3; KIAA1324L: KIAA1324-like; MGC4175: Mammalian Gene Collection 4175; CROT: carnitine O-octanoyltransferase; ABCB4: ATP-binding cassette, sub-family B (MDR/TAP), member 4; ABCB1: ATP-binding cassette, sub-family B (MDR/TAP), member 1.