

# Epistatic interactions govern chemically-induced lung tumor susceptibility and *Kras* mutation site in murine C57BL/6J-ChrA/J chromosome substitution strains

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Cancer susceptibility results from interactions between sensitivity and resistance alleles. We employed murine chromosome substitution strains to study how resistance alleles affected sensitive alleles during chemically-induced lung carcinogenesis. The C57BL/6J-Chr<sup>A/J</sup> strains, constructed by selectively breeding sensitive A/J and resistant C57BL/6J (B6) mice, each contain one pair of A/J chromosomes within an otherwise B6 genome. *Pas1*, the major locus responsible for this differential strain response to urethane carcinogenesis, resides on Chr 6, but C57BL/6J-Chr<sup>A/J</sup> mice (hereafter CSS-6) developed few tumors following a single urethane injection, which demonstrates epistatic interactions with other B6 alleles. CSS6 mice developed dozens of lung tumors after chronic urethane exposure, however, indicating that these epistatic interactions could be overcome by repeated carcinogen administration. Unlike A/J, but similar to B6 mice, CSS6 mice were resistant to lung carcinogenesis induced by 3-methylcholanthrene (MCA). Tumor multiplicity increased if BHT administration followed urethane exposure, showing that a Chr 6 gene(s) regulates sensitivity to chemically-induced tumor promotion. Unlike A/J tumors (predominantly codon 61 A→T transversions), *Kras* mutations in tumors induced by urethane in CSS-6 mice were similar to B6 tumors (codon 61 A→G transitions). DNA repair genes not located on Chr 6 may determine the nature of *Kras* mutations. CSS-6 mice are a valuable resource for testing the ability of candidate genes to modulate lung carcinogenesis.

Lung cancer is the leading cause of cancer death in both men and women in the USA, with 200,000 new cases of lung cancer estimated to be diagnosed this year. The dismal 15% survival rate over 5 years indicates that only 30,000 of these patients will be alive in 2014.<sup>1</sup> Smoking contributes to 85–90% of all lung cancers, but non-smoking-related lung cancer, which is more common in women than men, is the 7<sup>th</sup> leading cause of cancer death.<sup>2</sup> Since only ~15% of smokers develop lung cancer, genetic factors presumably contribute to disease susceptibility.

Recent lung cancer familial linkage studies delineated regions on human Chromosomes 6 and 12 that contributed to the risk of lung cancer, chronic obstructive pulmonary disease (COPD), and diminished lung function.<sup>3,4</sup> Chronic pulmonary inflammation predisposes toward lung cancer, and

several chromosomal sites in mice that determine responsiveness to pro-inflammatory pneumotoxins correspond to loci containing lung cancer susceptibility genes.<sup>5</sup>

Mice develop pulmonary adenocarcinoma (AC) that is histologically and genetically similar to human AC within a relatively short time span (6–42 wks) in response to chemical carcinogens<sup>6</sup> and within 2–36 wks in response to genetic induction with oncogenes such as *Kras*.<sup>7–9</sup> A/J mice are particularly sensitive to carcinogen-induced lung tumorigenesis, and develop spontaneous lung tumors during their lifespan.<sup>10</sup> Conversely, C57BL/6J (B6) mice are resistant to both carcinogen-induced and spontaneous lung tumor formation.<sup>10,11</sup> Mapping studies involving Mendelian crosses,<sup>12</sup> recombinant inbred strains<sup>13,14</sup> and advanced intercross lines<sup>15</sup> using these two strains have yielded much information about lung cancer susceptibility. Among the many susceptibility genes mapped using A/J and B6, the *Pas1* (*Pulmonary adenoma susceptibility 1*) locus on Chr 6 that contains six genes is responsible for >60% of the differential susceptibility to lung carcinogenesis in these two strains.<sup>16</sup> Polymorphisms in two candidate genes in this locus, *Kras* and *Cascl*,<sup>17</sup> contribute directly to this difference in susceptibility, while polymorphisms in the *Lrmp* gene that maps to this site in mice are associated with survival in human lung cancer patients.<sup>18</sup> Genome-wide association studies, linkage disequilibrium analysis, new mouse

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