

Genetic variation of the natural killer gene complex has a role in lung cancer susceptibility

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Worldwide estimates indicate lung cancer is the leading cause of death among all cancers, while also being the most frequently diagnosed of new cancer cases (1). Non-small cell lung carcinoma is the most frequent subtype. Exposure to environmental carcinogens, such as radon and tobacco inhalation, is a major risk factor for developing lung cancer. Familial heredity also increases an individual's risk, although the impact of genetic factors on the development of lung cancer remains unclear, especially within the context of immune response. Innate and adaptive immune systems may inhibit or enhance carcinogenesis depending on the recruitment of specific immunocompetent cells (2). Identifying specific mutations or variants directly affecting immune response to carcinogens, or developing tumors, remains difficult. Using genome wide association, candidate gene, and linkage approaches, a majority of the discovered loci associated with cancer pathogenesis are directly involved in tumor formation (i.e., cell proliferation and DNA damage pathways), rather than immune response. Animal studies are beginning to unravel the intersection of immunogenetics and carcinogenesis, and provide insight to disease vulnerability.

Few disease risk alleles related to immune response have been identified for specific cancers (3). Obtaining the required statistical power for identifying causal variants at particular stages of tumor development is typically unfeasible in human studies, especially if the genetic loci are of small effect. Animal studies have mapped almost 300 genetic loci that contribute to cancer pathogenesis (4,5), yet very few are verified (6,7). In a recent issue of *Cancer Research*, Kreisel *et al.* (8) reports novel evidence that a polymorphic region in the natural killer gene

complex (NKC) contributes to strain variability in urethane-induced lung cancer, an experimental paradigm often used to model multistage human lung cancer (9). Further, they provide insight into potential mechanisms of enhanced cytotoxic abilities of NK cells in lung cancer resistant mice.

Urethane-induced lung cancer varied substantially between three inbred mouse strains, AJ, 129SvEvTac [129], and C57BL/6J (B6). AJ displayed dramatically increased tumor number, size, and burden, while 129 were moderately susceptible and B6 were resistant. Kreisel and colleagues performed an elegant experiment to determine whether hematopoietic cells contribute this strain variability. AJ mice were injected with bone marrow derived cells from B6 mice prior to urethane injection, and vice versa. Tumor burden was significantly reduced in AJ/B6 bone marrow chimera mice, while B6/AJ mice showed increased tumor burden, although the effect was less dramatic. Replenishment of immunocompetent cells, or stimulation of the innate immune system, by hematopoietic progenitor cells may specifically increase immunosurveillance efficiency in vulnerable mice (10), rather than actions on adaptive responses.

Indeed, inflammatory activation of neutrophils, macrophages, or other monocytes was similar among the three strains. Even immunodeficient mice, (B6Rag^{-/-} and athymic B6 nude mice), were equally as susceptible to carcinogenesis, and depletion of T lymphocytes in AJ mice failed to have any effect on tumor burden or grade. In resistant B6 mice, depleting NK cell populations led to pronounced increases in tumor incidence (100% of mice) and tumor progression. Despite increased carcinogenesis in 129 mice compared to B6 mice, NK cell cytotoxicity was similar between these two strains, and essentially absent in AJ mice, indicating other factors influence lung carcinogenesis in these strains. The time course of innate and adaptive immune response to urethane also depends on strain and tissue microenvironment (11).

The NKC contains many genes involved in activating and inhibiting NK cell mediated lysing of tumor cells (12), and polymorphisms in this region could account for strain variability in immunosurveillance and consequently tumor formation (13-15). Interestingly, Balb/c mice, which share NKC homology with

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129 and AJ mice, also have reduced NK cell cytotoxicity (8). Although other immune mechanisms, such as NF- κ B activated inflammation, are involved in acute urethane response in these mice (16), it further implicates the NKC in lung cancer cytotoxicity. Kreisel and colleagues reported evidence for a novel role of the NKC in strain specific resistance to urethane-induced lung cancer. NKC allelic variation appears to, at least in part, contribute to cancer susceptibility phenotypes between 129 and B6 mice. Surprisingly, the authors found a substrain of Rag^{2-/-} mice, which are deficient in both mature B and T lymphocytes, was incompletely backcrossed to 129. In depth genomic analysis identified that this substrain was B6 congenic for the NKC locus (129/SvEv.B6-NKC Rag^{2-/-}), providing a model to explore the NKC from a resistant B6 on a moderately susceptible 129 background. As anticipated, mice congenic with B6 NKC locus displayed increased NK cell cytotoxicity and significant reductions in tumor burden compared to Rag^{2-/-} mice on a complete 129 background (129SvEvRag^{2-/-}). Thus, it appears B6 NKC alleles are protective against certain types of lung cancer, and T lymphocytes appear to contribute little to this phenotype.

As mentioned, the highly conserved NKC contains many genes critical for modulating NK cell activity, such as *Nkrp1* and *Nkg2* isoforms, and *CD69* and *CD94* (12). Among inbred mouse strains, the NKC is highly polymorphic (13). Allele variants in an array of NKC genes are mapped to NK cell phenotypic variation among inbred mouse strains (17). *In vitro* experiments from Kreisel *et al.* (8), demonstrated NKG2D receptors, a gene within the NKC (*Klrk1*), is necessary for enhanced NK cell lysis of lung cancer cells in resistant B6 mice. It is plausible polymorphisms in *Klrk1* underlie strain variability in lung cancer cytotoxicity and susceptibility.

Dr. Kreisel and colleagues chose to explore genetic variation between AJ, 129, BALB/cJ and B6 mice using haplotype and sequence analyses. There is a high degree of haplotype similarity between AJ and BALB/cJ mice throughout the NKC (128-132 Mb). Intriguingly, although other spans in the NKC show a similar pattern, haplotypes specifically aligning to the coding region of *Nkg2d* are similar among AJ, 129, and BALB/cJ strains, yet contrast B6. Moreover, comparative sequence analysis indicates there are numerous single nucleotide polymorphisms (SNPs) present in lung cancer susceptible and NK deficient AJ, 129, and BALB/cJ mice at the NKC locus, including many non-synonymous coding SNPs in *Ly49* (*Klra*), *Ly55* (*Klrb1*), and *NKG2* (*Klr*) isoforms, among others. In regards to *Nkg2d* (*Klrk1*), only two non-synonymous coding SNPs segregate in AJ and BALB/cJ strains. Isolating the allele and related immune-cancer phenotype among multiple strains could be difficult, but also worthwhile, especially if it is determined the risk allele is associated with impaired NKG2D receptor activation. Targeting NKG2D receptors is being explored as a possible immunotherapy for specific cancers (18).

The impact of genetic diversity on immune response to carcinogenesis is largely unexplored. It has been laborious and cumbersome to identify novel genes, or gene modifiers associated with innate and adaptive immune response during acute and chronic carcinogenic exposure. Kreisel and colleagues (8) demonstrate the role of a highly polymorphic NKC in vulnerability to tumor development, and reiterates the fundamental immunosurveillance capabilities of NK cells during acute carcinogenic exposure. Similar studies using new mouse resources with high genetic diversity and phenotypic variation, the Collaborative Cross (19) and Diversity Outbred (20), should provide novel roles for immune genes in cancer pathogenesis. Various cancer therapies using these populations could also shed light on why treatment success varies between individuals with similar or different cancer subtypes. Teasing apart genetic mechanisms modulating immune response at different stages of tumor development and at specific tissues is necessary for translating to human populations.

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