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Mutational profiling of sporadic versus toxin-associated brain cancer formation: Initial findings using loss of heterozygosity profiling

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ABSTRACT

The role of environmental and occupational toxin exposure as a cause of or contributing factor for cancer development and progression is incompletely understood. A unique signature of specific mutational change to discriminate toxin-exposed from sporadic cancer is generally sought but not often encountered. We report an approach to better understand cancer causality based on the measurement of the cumulative DNA damage (via loss of heterozygosity) over a defined genomic region (chromosome 3) that is applicable to archival, fixative-treated tissue and cytology specimens of cancer. Our method was applied to (1) a cohort of 10 brain tumor subjects (9 gliomas, 1 hemangioblastoma) with potential exposure to chlorinated solvents and (2) a control cohort of sporadic brain cancer controls (7 gliomas, 1 hemangioblastoma). We show that brain tumors arising in potentially toxin-exposed subjects bear a significantly higher level of passenger LOH mutations compared to sporadic cancer controls. The methodology utilized tissue microdissection, PCR amplification and capillary electrophoresis (fragment analysis for LOH determination, DNA sequencing for specific point mutations), and examined a panel of 15 microsatellite markers distributed along both arms of chromosome 3 that aimed at capturing passenger mutational change accrued during stages of clonal expansion of neoplastic cells. This proof-of-principle study using mutational profiling for passenger LOH mutational damage provides support for the utility of this approach and further studies in order to differentiate between genotoxin-associated versus sporadic (unexposed) cancer development.

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Introduction

Interest in the role of environmental and occupational chemical exposure as a cause for cancer has recently received increased attention reflected in statements from national oversight bodies and international agencies (Reuben, 2010; Fontham et al., 2009). The International Association for Research on Cancer and other organizations list a wide range of chemical agents as having been proven to be carcinogenic in humans (category 1) (IARC, 1995, 2009; NTP, 2002). Many more compounds are suspected to be capable of cancer formation and await further evidence to establish whether they can be said to specifically cause cancer. In attempting to directly link a previous exposure to a specific carcinogen with human cancer formation, a common approach is to search for a mutated gene or locus (or a set of such mutations) that is unique to the agent that caused the cancer. Unfortunately a discriminating set of mutations of this type exists in only a small number of instances (Gouas et al., 2009; Zhang, 2010); more frequently, the specific mutations are not significantly different between the toxin associated and sporadic cancer subjects. Likewise, sporadic and toxin associated cancers cancer generally do not demonstrate differentiating characteristics in microscopic appearance. We investigated a novel molecular signature method to discriminate sporadic, and exposure-related cancer formation and report preliminary results on this proof of principle approach here.

Cancer is a multistep process of clonal expansion events that confer progressively greater growth advantage to an affected cell compared to that of unaltered or less damaged cells (Nowell, 1976; Sjöblom et al., 2006; Greenman et al., 2007; Wood et al., 2007; Olivier et al., 2004; Bignell et al., 2010; Vineis et al., 2010; Pleasance et al., 2010). Clonal expansion occurs in response to DNA damage to specific oncogenes and tumor suppressor genes normally responsible for growth regulation. Mutations that result in clonal

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expansion are termed "driver" mutations in that the dysregulation of growth regulation resulting from these mutations "drives" cancer development and progression (Bozic et al., 2010; Haber and Settleman, 2007; Salk and Horwitz, 2010). However, mutations are also frequently found in regions of the genome that do not harbor oncogenes or tumor suppressor genes (Bozic et al., 2010; Haber and Settleman, 2007; Salk and Horwitz, 2010). These latter mutations are termed "passenger" mutations, since they do not necessarily drive further neoplastic progression. As a tumor cell population clonally expands, the tumor cells will carry not only driver mutations affecting critical growth regulatory genes but also co-existing passenger mutations that are present in the genome of the affected cell at times of clonal expansion.

Based on animal studies and human research, broad classes of chemical agents have been shown to directly damage DNA, leading to mutations and cancer (Donahue et al., 2006; Ellinger-Ziegelbauer et al., 2009; Pfeifer and Besaratinia, 2009; Lin et al., 2006; Murthy and Testa, 1999; Dusinská et al., 2004; Andujar et al., 2010). Renal cell carcinomas (RCC) in particular are known to develop following exposure to high levels of chlorinated solvents (Caldwell and Keshava, 2006; Tabrez and Ahmad, 2009; Lock and Reed, 2006; Kelsh et al., 2010; Moore et al., 2010; Shiao, 2009; Brüning et al., 1997; Brauch et al., 1999, 2004; Wells et al., 2009) and such changes have been described in many forms of toxin associated human cancer.

In a pair of studies of industrial workers exposed to high ambient air levels of trichloroethylene and related chlorinated solvents and sporadic renal cell carcinomas, the investigators found that the microscopic appearance of the kidney cancers in the two cohorts were similar and could not be distinguished from each other (Brauch et al., 1999, 2004). At the DNA level, however, the investigators found three distinct differences in the von Hippel–Lindau (VHL) gene, known to be closely associated with renal cell carcinoma formation, pointing to increases in the numbers of mutations in a particular segment of the genome for toxic-exposed tumors. First, there was a dramatically higher overall incidence of point mutations in the exposed cohort than the unexposed (82% versus 10% respectively), whereas previous studies have found established that in RCC overall frequencies of occurrence of, VHL point mutations of are present in only 30–70% RCC (Banks et al., 2006; Clifford et al., 1998; Wells et al., 2009). The second difference was that point mutations in the distal portion of exon 1 of the VHL gene were more common in exposed tumors than in sporadic tumors and in particular a 'hot spot' site of damage at codon 81 occurred only in exposed tumors. Third, multiple point mutations in the VHL gene were observed in exposed tumors but not in sporadic RCC. The third observation was particularly noteworthy since the occurrence of multiple mutations is uncommon in sporadic RCC – supplemental data from van Houwelingen et al. (2005) shows less than 4% of tumors having multiple mutations. However, this third observation is consistent with more intense DNA damage over a short time period from exposure to high levels of a toxin.

Two analogous studies of non-small cell lung cancer arising in subjects with significant levels of asbestos exposure lend further support for the concept of increased levels of genome-wide damage in toxin-associated cancers. In one study, such cancers arising in asbestos exposed individuals displayed a significantly higher rate of deletion of the p16 (CDKN2A) tumor suppressor gene known to act as a driver mutation in lung cancer as well as in many other forms of cancer. The other study found increased numbers of DNA strand breaks and chromosomal breaks in individuals with high levels of exposure to asbestos (Dusinská et al., 2004).

The common theme among all these studies was widespread DNA damage in toxin-induced human tumorigenesis, which we believed would also likely result in an increased load of passenger mutational change. Since driver mutations are by definition found in all cancers, we hypothesized that increased DNA damage would be more easily detected in the overall numbers of passenger mutations from exposure-related cancers than from sporadic cancers.

In previous unpublished work, we attempted to extend the specific findings of increased numbers of VHL mutations in RCC exposed to trichloroethylene towards an overall increased level of passenger mutations on the same chromosome as VHL, chromosome 3. The unpublished work considered subjects with demonstrable occupational exposure to trichloroethylene as a degreaser, and found that a high level of passenger mutational change was indeed observed in these toxin-exposed cancers. The present study extends that work in two important ways. Firstly, analyzing tumors from a tissue bank with no reported exposure to chlorinated solvents permits examination of the expectation of low numbers of passenger mutations in cancers lacking toxic exposure. Secondly, the present study extends the molecular signature concept to a new cohort of patients potentially exposed via air or groundwater contamination to trichloroethylene and its degradation products as well as other chlorinated solvents and toxins.

Additionally, the inclusion of hemangioblastomas in the subject cohort offered an opportunity to examine the findings of exposure-related VHL mutations in a tumor related to RCC. Hemangioblastomas are generally indolent tumors of the central nervous system, and share a number of characteristics with RCC, including very similar microscopic appearance. Like RCC, hemangioblastomas occur frequently in people with von Hippel syndrome (Nielsen et al., 2011), which involves germline mutations of the VHL gene, and sporadic hemangioblastomas (i.e. no inherited VHL mutations) also manifest VHL mutations in 4–14% of cases (Kim and Kaelin, 2004; Woodward et al., 2007). Given the similarities between RCC and hemangioblastoma and the common relationship to the VHL it seemed reasonable to look for the toxin-associated VHL mutations found in RCC in hemangioblastoma.

Materials and methods

Study cohorts

Since 1993, an unusually high number of primary brain cancers (oligodendroglioma, glioblastoma multiforme) have been observed among the residents of McCullom Lake, IL. The elevated number of brain cancers has raised concern about the possibility of a cancer cluster in this region.

The cancers occurred among individuals residing in a neighborhood of McCullom Lake Village which lies a little over a mile south of a chemical facility in the vicinity. Previous studies by the US Environmental Protection Agency (US EPA) and the Illinois Environmental Protection Agency (IEPA), as well as internal studies by the facility, demonstrated that chlorinated solvents had contaminated groundwater within the facility, resulting in a plume that extends approximately a mile to the southwest from the facility. As a result, the US EPA determined that air and groundwater contamination posed health risks to people living in the vicinity.

Further studies performed found a region of air contamination completely enveloping McCullom Lake Village, and extending to the southern edge of the McCullom Lake, with airborne levels of vinyl chloride of between 0.08 and 0.2 $\mu g/m^3$. These studies also found a plume of deep groundwater contamination – distinct from that found the earlier EPA and IEPA studies – extending in a more southerly direction from the facility and crossing substantial parts of McCullom Lake Village (Craver and Northwest Herald Staff, 2007). On the basis of this risk of exposure we consider this group to be potentially, though not definitively, exposed; for simplicity we refer to this group as "exposure-related".

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