Arsenic, Stem Cells, and the Developmental Basis of Adult Cancer

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That chemical insults or nutritive changes during in utero and/or postnatal life can emerge as diseases much later in life are now being accepted as a recurring phenomenon. In this regard, inorganic arsenic is a multisite human carcinogen found at high levels in the drinking water of millions of people, although it has been difficult until recently to produce tumors in rodents with this metalloid. A mouse transplacental model has been developed where maternal exposure to inorganic arsenic either acts as a complete carcinogen or enhances carcinogenic response to other agents given subsequently in the offspring, producing tumors during adulthood. Similarly, human data now have emerged showing that arsenic exposure during the in utero period and/or in early life is associated with cancer in adulthood. The mouse arsenic transplacental model produces tumors or enhances response to other agents in multiple strains and tissues, including sites concordant with human targets of arsenic carcinogenesis. It is now believed that cancer often is a stem cell (SC)–based disease, and there is no reason to think cancer induced by developmental chemical exposure is any different. Indeed, arsenic impacts human SC population dynamics in vitro by blocking exit into differentiation pathways and thereby creating more key targets for transformation. In fact, during in vitro malignant transformation, arsenic causes a remarkable survival selection of SCs, creating a marked overabundance of cancer SCs (CSCs) compared with other carcinogens once a cancer phenotype is obtained. In addition, skin cancers produced following in utero arsenic exposure in mice are highly enriched in CSCs. Thus, arsenic impacts key, long-lived SC populations as critical targets to cause or facilitate later oncogenic events in adulthood as a possible mechanism of developmental basis of adult disease.

Key Words: arsenic; cancer; development; epigenetics; stem cells.

Inorganic arsenic naturally occurs in the environment often as a high-level contaminant of human drinking water (IARC, 2004). Being a metalloid, elemental arsenic has carbon-like qualities reflected in bonding characteristics but also characteristics that are more typical for transition metals. Arsenic is rapidly excreted, primarily through the urine (IARC, 2004). Inorganic arsenic, which exists in a trivalent (arsenite) or pentavalent (arsenate) form, undergoes biomethylation through specific methyltransferases that use S-adenosylmethionine (SAM) as the methyl donor, and metabolites include pentavalent or trivalent monomethylated and dimethylated arsenicals (Carter et al., 2003; IARC, 2004; Thomas, 2007). Mice and humans show generally similar arsenic metabolism, whereas rats have quite divergent circulating binding proteins (Carter et al., 2003; Lu et al., 2007; Naranmandura and Suzuki, 2008). Once inorganic arsenic reaches biological systems, it often changes valence spontaneously or actively between arsenite and arsenate. Hereafter, the terms arsenic and inorganic arsenic are applied to both arsenite and arsenate to reflect the uncertainty of their nature and quantity in biological systems.

Inorganic arsenic is considered a known human carcinogen with various target tissues, including the skin, urinary bladder, and lung (IARC, 2004, 2009). The liver, kidney, and prostate are now considered as potential human target tissues as well (IARC, 2009). Inorganic arsenic has, in fact, been long recognized as a human carcinogen (IARC, 1973, 1980, 1987, 2004, 2009). Yet, in rodents, it had proven difficult to induce tumors after inorganic arsenic exposure as a single agent, indicating rodents are likely insensitive to arsenic when compared with humans (Tokar et al., 2010a; Waalkes et al., 2007). Indeed, Carter et al. (2003) makes the point that for toxicity studies, it often takes 10–100 times the dose in humans for animals to manifest a similar toxic effect. In fact, clear and repeatable rodent evidence of complete carcinogenic effects after inorganic arsenic exposure was not available until the development of a transplacental model of arsenic carcinogenesis in mice where animals were exposed in utero via the dam’s drinking water and developed tumors in adulthood (Tokar et al., 2010b; Waalkes et al., 2003, 2004b, 2006a,b). In general, early life can be a period of high sensitivity to carcinogenesis induced by many chemicals, which are manifested as tumors in adulthood because of factors such as rapid
and global proliferative growth, organogenesis-related cell differentiation and apoptosis, and genetic imprinting in early life (Anderson et al., 2000; Anderson 2004; Birnbaum and Fenton, 2003; Waalkes et al., 2007), processes largely involving stem cells (SCs). This does not mean that early-life exposure is necessarily linked to early-onset childhood cancers (Anderson 2004), although this can happen. Thus, it appears that, although insensitive as to inorganic arsenic as adults, rodents display a propensity toward development of tumors after in utero exposure to arsenic (Tokar et al., 2010b; Waalkes et al., 2003, 2004b, 2006a,b). Although it has not been tested in transplacental models of exposure, dimethylarsinic acid given chronically in the water or feed of adult rats will consistently cause urinary bladder tumors (Arnold et al., 2006; Wei et al., 1999), indicating that adult rodents can be sensitive to arsenic carcinogenesis, too.

CANCER AS A DISEASE OF STEM CELLS

The idea that cancers may develop from a small subpopulation of cells originated over 100 years ago (Conheim, 1875; Durante, 1874; see Polyak and Hahn, 2005). Subsequent early studies built on this notion and provided evidence that only a rare population of tumor cells could recapitulate the original tumor when transplanted into a recipient animal (Bruce and Van Der Gaag, 1963; Furth and Kahn, 1937; Hamburger and Salmon, 1977; Hewitt, 1953; Ishibashi, 1950; Makino, 1956; Till and McCulloch, 1961). These rather remarkable studies were the first to propose the existence of cancer SCs (CSCs). But proof that tumors exist in a hierarchical structure, with CSCs residing at the apex of this structure and driving tumor formation and growth, came with the seminal CSC biology work by John Dick and colleagues (Bonnet and Dick, 1997; Lapidot et al., 1994). Since then, many studies have demonstrated the existence of CSCs in both solid tumors and hematologic malignancies (for reviews, see Lobo et al., 2007; O’Brien et al., 2010; Pardal et al., 2003; Visvader and Lindeman, 2008). These studies, coupled with the many shared characteristics between normal SCs and CSCs, provide strong, accumulating evidence that leads many to believe that cancer is a disease of SCs.

A SC is a clonogenic cell that, through differentiation, can regenerate all cell types of any tissue in the body, if an embryonic SC, or of all cell types of a given tissue, if an adult SC or “progenitor” cell (Pardal et al., 2003; Polyak and Hahn, 2005; Wicha et al., 2006). Nearly all tissues and organs contain small populations of these rare cells. SCs reside at the apex of a hierarchical structure in normal tissues and can divide to give rise to undifferentiated daughter cells or differentiate to form mature cell types (Fig. 1). SCs have several key fundamental characteristics that distinguish them from other cells, perhaps the most important being that of self-renewal (Pardal et al., 2003; Polyak and Hahn, 2005).

![FIG. 1. Numerous potential environmental factors can lead to the formation of CSCs from normal SCs and/or progenitor cells. Normal SCs have the ability to self-renew (circular arrow) and differentiate into transient amplifying/progenitor cells, which subsequently differentiate into various mature cell types. Different colors represent transit-amplifying cells dedicated to a different phenotypic pathway. Cancer SCs originate following genetic and/or epigenetic events in normal SCs and/or, possibly, progenitor cells. Cancer SCs also have the ability to self-renew and also to differentiate into a heterogenous tumor cell population, both characteristics which help to drive tumorigenesis. Question mark (?) indicates that the generation of CSCs from certain cell phenotypes is unresolved. (Adapted from Jordan et al., 2006.)](https://academic.oup.com/toxsci/article-abstract/120/suppl_1/S192/1619775)
the potential for self-renewal, which, with CSCs, gives rise to phenotypically similar tumorigenic cancer cells and drives carcinogenesis. Cancer SCs also possess the ability to differentiate, although aberrantly, which generates the vast cellular heterogeneity common in tumors. Cancer SCs express or show activated expression of many of the same cell surface markers and self-renewal markers as do normal SCs (Bomken et al., 2010; Lobo et al., 2007; O’Brien et al., 2010; Pardal et al., 2003; Visvader and Lindeman, 2008). Furthermore, a common set of signaling pathways (i.e., Wnt, Hedgehog, and Notch) that regulate SC maintenance, lineage determination, differentiation, etc., are dysregulated in many cancers (Lobo et al., 2007; Pardal et al., 2003; Reya et al., 2001), particularly in the putative CSC subpopulation. Finally, cancer is a multistep process requiring multiple “hits” for neoplastic transformation (Vogelstein and Kinzler, 1993). Often there is a long period between the events of initiation and eventual tumor formation. Therefore, a long-lived cell (SC) would be necessary to accumulate the hits necessary for transformation, which would point toward an already “immortal” SC.

Thus, emerging theory indicates that cancers may form in pluripotent SC populations and that the normal SC characteristics of quiescence, self-renewal, and conditional immortality would potentially supply a lifelong, latent neoplastic population after carcinogen attack (Kangsamaksin et al., 2007; Lobo et al., 2007; Pardal et al., 2003; Visvader and Lindeman, 2008). Fetal SCs are probably key targets in transplacental carcinogenesis (Anderson et al., 2000; Waalkes et al., 2007) and may impact fetal sensitivity based on relative abundance and high activity. During development, fetal SCs have several characteristics that would favor them as targets for carcinogens, although they may be no more inherently sensitive than adult SCs. In utero, for instance, fetal SCs are involved with intense global proliferative growth, differentiation for organogenesis, and have a higher relative abundance than in adults (Anderson et al., 2000; Wicha et al., 2006), all of which would create more targets for carcinogen interactions. Nonetheless, the identity of the normal cell or cells that initially acquire the lesions that then drive cancer remains elusive (Bomken et al., 2010; Perez-Losada and Balmain, 2003), regardless of the life stage of initiation. However, the idea that inorganic arsenic as a transplacental carcinogen may target fetal SCs (Tokar et al., 2010a; Waalkes et al., 2007, 2008) and impact SC behavior/function/number is really the convergence of two hypotheses. The first being that the fetal basis of adult disease is likely based in cells with long memories, such as SCs, that can be forced to a disease state much later and the second being that cancer is likely a SC-based disease.

THE DEVELOPMENTAL BASIS OF ADULT DISEASES

Background

Accumulating and compelling evidence indicates that events in utero or during early life impact the development of human disease during adulthood (Barker, 2007; Dolinoy et al., 2007a,b; Morley, 2006). Called by many the “Barker” hypothesis, this concept arose from multiple studies that correlated low birth weight with various adult-onset diseases, such as type-II diabetes, coronary heart disease, high blood pressure, and stroke (Morley, 2006). Implicit in this evolving theory is that undernutrition early in human life caused permanent changes in the perinate that then, in turn, subsequently caused or enhanced susceptibility to adverse effects, including overt disease, much later in life (Barker, 2007; Morley, 2006). Multiple epidemiologic studies support this finding of undernutrition in early-life–enhancing adult disease (Barker, 2007; Morley, 2006). The gap in time between the original insult (early life) and the time of expression of the adverse effect (adulthood) would necessarily mean that permanent but quiescent changes had occurred in the perinate that could be stimulated later in life to foster distortion of normal function that, in turn, leads to a disease state.

There is no real reason to suppose that the phenomenon of the developmental basis of adult disease would not extend beyond inappropriate nutrition as the “initiating” insult to chemical exposures. In fact, there is clear evidence that chemical exposures during perinatal life can have a remarkable impact on various disease states in adulthood. For example, there is reason to believe that developmental toxicant exposure may play a role in diabetes and obesity (Lassiter et al., 2008). Furthermore, perinatal exposure to physical or chemical agents has long been known to be carcinogenic in humans or rodents when they reach adulthood (Anderson et al., 2000; Tomatis, 1989). This includes development exposure to estrogenic compounds, which can cause cancer in both male and female offspring after in utero and/or developmental exposure (Anderson et al., 2000; Birnbaum and Fenton, 2003; Prins et al., 2007), as exemplified by diethylstilbestrol (DES), a known human transplacental carcinogen (Anderson et al., 2000). A variety of compounds collectively considered as endocrine disruptors are similarly associated with cancers after exposure during early development (Birnbaum and Fenton, 2003). Various animal experiments clearly indicate that in utero and/or early-life chemical exposure can either act as complete carcinogens or produce lesions that can be promoted by other agents into tumors later in life in a wide variety of target organs (Anderson, 2004; Anderson et al., 2000; Birnbaum and Fenton, 2003; Tokar et al., 2010a; Tomatis, 1989; Waalkes et al., 2007). It is important to realize that perinatal carcinogen exposure should not be presumed to be specifically or generally associated with childhood cancers, although these sorts of cancers do occur after perinatal exposures (Anderson, 2004).
Epigenetic and Other Mechanisms of the Developmental Basis of Adult Disease

It is thought that imprinted genes may be a key target in the perinate as these are likely “epigenetically labile” genes involved in the plasticity of the fetal genome (Dolinoy et al., 2007a,b; Waterland and Jirtle, 2004). It is suspected that the nutritional status of the perinate at critical time points in development produces a lasting influence on gene expression via epigenetic mechanisms, including changes in DNA methylation or chromatin packaging (Dolinoy et al., 2007a,b; Waterland and Jirtle, 2004). For nutritionally associated disease, these epigenetic effects thereby form a key link between nutrition and early modification of the genome, which is manifested later in life (Waterland and Jirtle, 2007). For example, DNA methylation at CpG dinucleotides occurs nonrandomly and is a major postsynthetic modification that clearly helps control gene expression capacity (Dolinoy et al., 2007a,b; Waterland and Jirtle, 2004). The critical cofactor in most cellular methylation reactions is SAM, which is used for DNA methylation by various mammalian DNA methyltransferases producing S-adenosylhomocysteine (SAH) after donation of the methyl group (Coppin et al., 2008; Dolinoy et al., 2007b; Loenen, 2006). Small changes in the levels of methyl-donating micronutrients, like choline, folic acid, cobalamin, and betaine, that help reestablish SAM levels (Coppin et al., 2007a,b; Loenen, 2006). For nutritionally associated disease, these epigenetic effects thereby form a key link between nutrition and early modification of the genome, which is manifested later in life (Waterland and Jirtle, 2007). For example, DNA methylation at CpG dinucleotides occurs nonrandomly and is a major postsynthetic modification that clearly helps control gene expression capacity (Dolinoy et al., 2007a,b; Waterland and Jirtle, 2004). The critical cofactor in most cellular methylation reactions is SAM, which is used for DNA methylation by various mammalian DNA methyltransferases producing S-adenosylhomocysteine (SAH) after donation of the methyl group (Coppin et al., 2008; Dolinoy et al., 2007b; Loenen, 2006). Small changes in the levels of methyl-donating micronutrients, like choline, folic acid, cobalamin, and betaine, that help reestablish SAM levels (Coppin et al., 2007a,b; Waterland and Jirtle, 2004). It is thought that the altered dietary availability of methyl donors and cofactors could influence DNA methylation during critical periods of development (Waterland and Jirtle, 2004). DNA-associated histone protein acetylation and methylation modify gene expression in a complex fashion by altering chromatin packaging (Dolinoy et al., 2007a,b; Waterland and Jirtle, 2004) and represent additional epigenetic factors that are likely involved at the molecular level in the developmental basis of adult disease. On a different level, it appears there is a possible role for mesenchymal SCs, as their reaction is altered, possibly through mutation response, at a key time in the developmental causation of valvular heart disease (Markwald et al., 2010).

It is noteworthy that arsenic also undergoes enzymatic biomethylation using SAM as the methyl donor and a specific methyltransferase (Coppin et al., 2008; Thomas, 2007). It appears that arsenic biomethylation is increased in pregnancy and therefore might be under, at least partial, control of estrogens (Tseng, 2009). The monomethylated trivalent product of arsenic biomethylation, methylarsanonic acid, appears to be the most toxic species of arsenic metabolism and may be the ultimate genotoxicant (Thomas, 2007). Arsenic biomethylation occurs in some, but not all, target cell types of arsenic carcinogenesis, and, interestingly, in those cells that do not methylate arsenic, there is no evidence of oxidative DNA damage (Kojima et al., 2009), what is thought to be a primary genotoxic mechanism for inorganic arsenic to act as a carcinogen (Hei and Filipic, 2004; Kitchin and Conolly, 2010). This creates the distinct possibility that cells not capable of methylating arsenic, as they gain a malignant phenotype, likely do so by nongenotoxic, epigenetic mechanisms (Coppin et al., 2008; Kojima et al., 2009). In fact, cells that methylate the metalloid and cells that do not methylate the metalloid will both show DNA hypomethylation during oncogenic transformation (Benbrahim-Tallaa et al., 2005; Coppin et al., 2008; Zhao et al., 1997). This is perhaps because of activation of an efflux system that extrudes arsenic as a glutathione trimer (Leslie et al., 2004), part of an acquired arsenic adaptation mechanism that occurs concurrently with malignant transformation and requires diversion of homocysteine (from SAH) to glutathione (Coppin et al., 2008). However, this effectively limits SAM recycling even in target cells that do not use it directly for arsenic methylation and thereby allows DNA hypomethylation even in cells that do not biomethylate the metalloid (Coppin et al., 2008). In fact, arsenic has been shown to vary across different gene expression in concert with hypomethylation of genomic or gene-specific DNA in vitro and in vivo (Benbrahim-Tallaa et al., 2005; Chen et al., 2004; Okoji et al., 2002; Reichard et al., 2007; Waalkes et al., 2004a; Xie et al., 2004; Zhao et al., 1997). Indeed, on several occasions, this has been shown with developmental exposure to transplacental carcinogenic doses in rodents (Waalkes et al., 2004a; Xie et al., 2004). Others have shown that both hypo- and hypermethylation of DNA can be observed after arsenic exposure (Boellmann et al., 2010; Jensen et al., 2009). Regardless, it is clearly possible that arsenic can have epigenetic effects based on DNA modification with exposure during development. Theoretically, epigenetic mechanisms might be viewed as requiring a minimal level to be active and thus have a threshold, although there is no direct evidence of this either way with arsenic exposure.

Genotoxic effects also appear possible with inorganic arsenic (Hei and Filipic, 2004; Kitchin and Conolly, 2010; Kojima et al., 2009). It is clearly possible, in the respect of cancer, for genotoxic events to lie dormant for a long time and then emerge at a much later time point. Selected cell populations that have the longevity to carry the molecular lesion to a later period would be the most likely repository, and this again points toward SCs.

DEVELOPMENTAL STUDIES AND ARSENIC CARCINOGENESIS

Mouse Bioassays

A relatively recent series of studies have been performed in which various strains of mice were exposed to inorganic arsenic via the maternal drinking water (Tokar et al., 2010b;
Waalkes et al., 2003, 2004b, 2006a,b, 2008). The first study exposed pregnant C3H mice orally to sodium arsenite from gestation day (GD) 8 to 18 with no additional exposures to arsenic or other compounds (Waalkes et al., 2003). Subsequent studies used other strains (CD1 and Tg.AC) and/or followed the in utero arsenic exposure with exposure of the offspring to tumor promoters (12-O-tetradecanoyl phorbol-13-acetate (TPA)) or estrogen-like compounds (DES and tamoxifen [TAM]) post partum to enhance tumor response (Tokar et al., 2010b; Waalkes et al., 2004b, 2006a,b, 2008).

In the first transplacental exposure study, pregnant C3H mice were exposed to 0, 42.5, and 85 ppm arsenic (as sodium arsenite) in the drinking water from GD 8 to 18 and allowed to give birth (Waalkes et al., 2003). After weaning, the offspring were observed for tumor development over 74–90 weeks (Waalkes et al., 2003). No additional arsenic was given to the offspring during the rest of the study. The levels of arsenic used in this and other transplacental studies (Tokar et al., 2010b; Waalkes et al., 2003, 2004b, 2006a,b, 2008) did not alter dam weights, dam drinking water consumption, litter size, newborn weights, or weanling weights or induce any signs of overt toxicity during exposure (Waalkes et al., 2003). In utero arsenic-exposed female offspring developed dose-related increases in lung adenocarcinoma, ovarian tumors, and also dose-related uterine and oviduct preneoplasias. After in utero arsenic exposure, male offspring showed dose-related increases in incidence of hepatocellular adenoma, hepatocellular carcinoma (HCC), and adrenal cortical adenoma (Waalkes et al., 2003). The short exposure period required for the carcinogenic delayed response (10 days) seen in this study indicates a high sensitivity. In addition, these data provided evidence that inorganic arsenic initiated long-lasting events in utero that manifested as cancer in adulthood. In fact, with the rapid clearance of arsenic in mice (Waalkes et al., 2007), it is unlikely that substantial amounts of the intentional treatment arsenic remain in the adults that were briefly exposed during gestation. Furthermore, these data indicate that continuously elevated exposure is not required for inorganic arsenic to be carcinogenic, and exposure only during this key period of early life is sufficient for a tumor response (Waalkes et al., 2003).

The external doses (42.5 and 85 ppm arsenic in the drinking water) used in this (Waalkes et al., 2003) and subsequent studies (Tokar et al., 2010b; Waalkes et al., 2003, 2004b, 2006a,b, 2008), although well tolerated by the animals using multiple metrics, have been labeled as “high.” However, it is very important to keep in mind the point made by Carter et al. (2003) that for similar toxic effects from arsenic to be manifested in animals, including mice, it often takes between 10- and 100-fold higher external doses when compared with humans. Although this principle was not applied to cancer (Carter et al., 2003), there is no reason to suspect that it would not extend to this toxic endpoint. In fact, although the external doses in the drinking water used in these mouse transplacental studies are between 100 and 200 times greater than that which are seen with human environmental drinking water exposures (IARC, 2004), fetal mouse blood arsenical levels in these transplacental studies are similar to what occur with human arsenic exposure (Devesa et al., 2006; Pi et al., 2002; Waalkes et al., 2007).

The skin is an important target site of arsenic carcinogenesis (IARC, 2004, 2009). Thus, a second study (Waalkes et al., 2004b) tested the hypothesis that in utero arsenic exposure might initiate events in the fetal skin but would still require postnatal promotion to produce skin cancer. Here, pregnant C3H mice received water with arsenite (0, 42.5, or 85 ppm) from GD 8 to 18, and, to try to promote any skin cancers initiated by fetal arsenic exposure, the offspring were exposed to TPA (topical, shaved back skin 2 μg, twice/week) from 4 to 25 weeks of age (Waalkes et al., 2004b). Despite the TPA exposure, fetal arsenic exposure with or without TPA did not impact skin tumors (Waalkes et al., 2004b). The C3H mouse is not particularly sensitive to skin carcinogens. However, like the first transplacental arsenic study (Waalkes et al., 2003), the male offspring developed dose-related, arsenic-induced HCC and adrenal tumors, independent of TPA (Waalkes et al., 2004b). Similarly, female offspring showed ovarian tumors and uterine and oviduct hyperplasias with arsenic independent of TPA (Waalkes et al., 2004b). TPA promoted liver tumors (females) and lung tumors (both sexes) in arsenic-treated animals, although not always in an arsenic dose-related fashion. Thus, arsenic did not initiate skin tumors in the C3H mouse fetus, but arsenic did appear to initiate some internal tumors that could be promoted by TPA (Waalkes et al., 2004b).

The consistent pattern of adult tumors/hyperplasias that developed after fetal arsenic exposure (i.e., liver, ovary, adrenal, uterus, and oviduct; Waalkes et al., 2003, 2004b) is also targets of broad range or tissue-selective carcinogenic estrogens (Anderson 2004; Birnbaum and Fenton, 2003; Waalkes et al., 2007). Because of this estrogenscan spectrum of tumors, we hypothesized that arsenic might induce aberrant estrogen signaling as part of a carcinogenic mechanism. Indeed, estrogens are linked to HCC, and we found hepatic overexpression of estrogen receptor-α (ER-α) and estrogen-regulated/linked genes in adult male C3H mice bearing HCC resulting after fetal arsenic exposure (Waalkes et al., 2004a). This ER-α overexpression also occurred in liver biopsies from adult humans with skin lesions typical of heavy arsenic intake (Waalkes et al., 2004a). To see if stimulation of aberrant estrogen signaling would aggravate the oncogenic patterns put into place by fetal arsenic exposure, pregnant CD1 mice were given arsenic (0 or 85 ppm) in the drinking water from GD 8 to 18 and were allowed to give birth, and female (Waalkes et al., 2006b) or male (Waalkes et al., 2006a) offspring were treated with DES or TAM by repeated sc injections on post partum days 1–5. Fetal arsenic alone in female offspring induced some urogenital system tumors (UST; mostly benign ovarian and uterine tumors) and adrenal cortical adenoma. DES alone induced some UST tumors (primarily
The male offspring from these same mothers showed that arsenic alone increased liver tumors, lung adenocarcinoma, and adrenal cortical adenoma as well as causing renal cystic tubular hyperplasia (Waalkes et al., 2006a). Compared with fetal arsenic alone, arsenic followed by DES synergistically increased liver tumor incidence (Waalkes et al., 2006a). Although the treatments singly did not impact urinary bladder tumors, arsenic plus TAM increased urinary bladder transitional cell tumors (papilloma and TCC). Bladder proliferative lesions (tumors + hyperplasias) were increased by fetal arsenic plus TAM or fetal arsenic plus DES compared with control or the various individual treatments. Urinary bladder tumors and HCC induced by arsenic plus TAM and/or DES overexpressed ER-α, indicating that aberrant estrogen signaling may be a factor in the enhanced response. Thus, fetal arsenic alone induced tumors of the liver, lung, and adrenal in male CD1 mice (Waalkes et al., 2006a). Fetal arsenic initiated urinary bladder TCC formation in male offspring with postnatal TAM treatment and increased urinary bladder proliferative lesions if combined with TAM or DES in male offspring (Waalkes et al., 2006a).

Thus, inorganic arsenic as a transplacental carcinogen duplicated or influenced tumor formation at several known or possible human target sites (i.e., lung, liver, and urinary bladder). There are also data indicating that inorganic arsenic will impact human skin SC population dynamics in vitro by blocking differentiation pathways (Patterson and Rice 2007; Patterson et al., 2005). In this regard, keratinocyte SCs (KSCs) are considered a critical target in skin carcinogenesis (Humble et al., 2005). Therefore, we studied the effects of fetal arsenic exposure in Tg.AC mice (Waalkes et al., 2008), a strain sensitive to skin carcinogenesis by way of activation of the v-Ha-ras transgene, which likely resides in KSCs (Humble et al., 2005; Trempus et al., 2003, 2007). After fetal arsenic exposure via the maternal system (0–85 ppm arsenite; GD 8–18), the offspring received topical TPA from weaning through adulthood at all major probable (lung, urinary bladder, and skin) and most possible target sites (liver and kidney) of arsenic carcinogenesis in humans (IARC, 2004, 2009), with the single exception of the prostate (Tokar et al., 2010b; Waalkes et al., 2003, 2004b, 2006a,b, 2008). The mouse may be a poor model for prostate carcinogenesis, which could be one reason an animal model is yet to be developed for arsenic carcinogenesis in the prostate (Benbrahim-Tallaa and Waalkes, 2008). Nonetheless, this is a truly remarkable tissue concordance.

### EARLY-LIFE ARSENIC EXPOSURE AND CANCER IN HUMANS

Various human studies now point to an association between early inorganic arsenic exposure and cancer later in life. People living in a specific region in Chile, known as administrative
Region II, in 1958 experienced a rapid onset of high arsenic exposure in the drinking water that increased levels in major cities up to 870 µg/l (ppb) from approximately 100 ppb (Marshall et al., 2007; Yuan et al., 2010). The major cities in this region include Antofagasta and Mejillones, which both reflected this change to high inorganic arsenic levels in the drinking water (Marshall et al., 2007; Smith et al., 2006; Yuan et al., 2010). The reason the drinking water arsenic became so rapidly elevated was that municipal drinking waters were supplemented in 1958 with water from rivers with naturally high inorganic arsenic levels, approaching 1000 ppb arsenic (Liaw et al., 2008; Marshall et al., 2007; Smith et al., 2006). Thus, rivers containing high inorganic arsenic levels were used to supplement the municipal drinking water and, e.g., the arsenic level in Antofagasta suddenly went from 90 to 870 ppb arsenic (Liaw et al., 2008). In addition, there are very few individual water supplies, making municipal sources with high arsenic the main source of drinking water in this area. In 1971, a treatment plant was installed that markedly reduced the drinking water arsenic levels in the area, as, for instance, seen with the drinking water of Antofagasta which suddenly dropped to a level of about 110 ppb arsenic (Liaw et al., 2008). Thus, the data on persons living in this area have provided a unique scientific resource for the study of diseases stemming from exposure to inorganic arsenic in the drinking water, including the onset and latency of arsenical-induced cancer (Liaw et al., 2008; Marshall et al., 2007; Smith et al., 2006; Yuan et al., 2010). Importantly, because this is a particularly large group with well-defined exposure (Marshall et al., 2007), this distinctive exposure scenario offers the ability to assess how developmental inorganic arsenic exposure has impacted adult cancer mortality. Indeed, in groups from this area, there are at least four separate studies that indicate that early-life exposure to inorganic arsenic is associated with human cancer in adulthood, including cancers of the lung (Marshall et al., 2007; Smith et al., 2006), liver (Liaw et al., 2008), and kidney (Yuan et al., 2010). The lung is considered a clear target site of arsenic carcinogenesis in humans, whereas the liver and kidney are considered possible target sites (IARC, 2004, 2009).

In this regard, Smith et al. (2006) compared the standardized mortality ratio (SMR) in Antofagasta, Chile, between 1989 and 2000 with the rest of Chile. They focused on subjects who were born during 1958–1970 (receiving in utero and childhood exposure) or just before the peak arsenic exposure period (1950–1957; receiving childhood exposure) and who were between 30 and 49 years old at the time of death by lung cancer. The SMR for lung cancer was 7.0 for people exposed in early childhood and 6.1 for people born during the high-exposure period with probable in utero and early childhood arsenic exposure. The authors concluded that exposure to inorganic arsenic in utero and/or during early childhood greatly increased pulmonary cancer in young adults (Smith et al., 2006). The latter exposure time included both males and females (Smith et al., 2006). In a much larger study, Marshall et al. (2007) looked at lung cancer mortality from all of Region II, which includes the city of Antofagasta. They found that early-life exposure (i.e., childhood and adolescence) in males results in remarkably high lung cancer rates in adulthood (Marshall et al., 2007), confirming, in part, their earlier work (Smith et al., 2006). They did not look at birth cohort that would include in utero exposure (Marshall et al., 2007).

A study compared childhood liver cancer mortality rates in Region II occurring during 1950–2000 with those of a control administrative Region (Region V) in Chile with known low arsenic levels in the drinking water (Liaw et al., 2008). Although the cases of childhood (< 19 years of age) liver cancer mortality were small (total eight), for persons exposed to arsenic as young children (i.e., born between 1950 and 1957), the relative risk (RR) of liver cancer mortality for males born during this period was 8.9 and for females was 14.1. The fact that large exposed and unexposed populations were

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<td>Male and female Tg.AC</td>
<td>Arsenic plus TPA</td>
<td>Waalkes et al. (2008)</td>
</tr>
<tr>
<td>Urinary bladder tumors</td>
<td>Male CD1</td>
<td>Arsenic plus TAM</td>
<td>Waalkes et al. (2006a)</td>
</tr>
<tr>
<td>Vaginal tumors</td>
<td>Female CD1</td>
<td>Arsenic plus DES</td>
<td>Waalkes et al. (2006b)</td>
</tr>
<tr>
<td>Renal hyperplasia</td>
<td>Male CD1</td>
<td>Arsenic alone</td>
<td>Waalkes et al. (2006a)</td>
</tr>
<tr>
<td>Oviduct and uterine hyperplasia</td>
<td>Female C3H, female CD1, and female Tg.AC</td>
<td>Arsenic alone</td>
<td>Tokar et al. (2010b) and Waalkes et al. (2003, 2004b, 2006b)</td>
</tr>
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Note. Selected lesion only including primary tumors but not all hyperplastic lesions. See individual studies for lesion incidence and treatment details.
studied, the high magnitude of the RRs, the robust \( p \) values involved, and the consistency between genders all suggested to the authors that these findings are not because of chance or bias (Liaw et al., 2008). These data suggest that exposure to arsenic in drinking water during early childhood may result in increased liver cancer mortality at an early age (Liaw et al., 2008). The number of cases of liver cancer mortality was too small with in utero exposure combined with childhood exposure (i.e., persons born between 1958 and 1970) to draw any conclusions (Liaw et al., 2008).

In very recent work, Yuan et al. (2010) analyzed data from Region II in Chile for kidney cancer deaths. Focusing on two cities with particularly high exposure (Antofagasta and Mejillones), they found that early-life arsenic exposure, which included both in utero and childhood exposures (birth during the period of 1950–1970), resulted in markedly higher kidney cancer mortality rates in young adults aged 30–39 years (Yuan et al., 2010). In fact, compared with the rest of Chile, the SMR for kidney cancer after early-life arsenic exposure in males aged 30–39 was 5.6, in females was 9.5, and combined was 7.1 (Yuan et al., 2010). This compared with the SMRs for kidney cancer in arsenic-exposed persons born before 1950 (having baseline in utero and much less childhood arsenic exposure) of 2.7 for males, 3.9 for females, and 3.1 for combined, rates which run less than half of those with early-life arsenic exposure (Yuan et al., 2010).

Taken together, these data from Chile provide evidence that environmental exposure to inorganic arsenic in the drinking water during the in utero and childhood developmental life phases can be associated with cancer during adulthood in humans and may be a period of high sensitivity for exposure based on the remarkable tumor rates (Liaw et al., 2008; Marshall et al., 2007; Smith et al., 2006; Yuan et al., 2010). The target sites in these human studies include the lung, kidney, and liver (Liaw et al., 2008; Marshall et al., 2007; Smith et al., 2006; Yuan et al., 2010). Interestingly, we find that in utero arsenic exposure in mice induces or initiates tumors or preneoplastic lesions in the lung, liver, and kidney in the offspring as they become adults (Waalkes et al., 2003, 2004b, 2006a), concordant with the targets in these human studies (Liaw et al., 2008; Marshall et al., 2007; Smith et al., 2006; Yuan et al., 2010). It is important to note that inorganic arsenicals for many years were not considered to be clearly carcinogenic in rodents, although the human data on arsenic as a carcinogen were considered unequivocal, first from industrial exposures and later from environmental exposures (IARC, 1973, 1980, 1987, 2004). The emergence of consistent data resulting from early-life arsenic exposures in mice resulting in tumors in adulthood, including tumors concordant with human early-life exposure studies (Waalkes et al., 2003, 2004b, 2006a), strongly supports the early-life period as a time of high sensitivity to arsenic carcinogenesis.

Another important human data set has just recently emerged that was because of a tragic poisoning incident in Japan from consumption of arsenic-contaminated powdered milk in 1955 (Yorifuji et al., 2010). This study concerns persons from Okayama where it is thought that over 2000 infants or children were poisoned or died because of consumption of this heavily arsenic-contaminated milk powder, and although this was less than 2% of the total Japanese population at the time, this may represent a total of 17% of the affected children (Yorifuji et al., 2010). The surviving population has been carefully followed and compared with a population born after poisoning (Yorifuji et al., 2010). Although the mortality from total cancer and lung cancer was not elevated, both skin and liver cancers are increased in survivors of the arsenic poisoning (Yorifuji et al., 2010). Unexpectedly, pancreatic cancers and leukemia are also elevated by early-life arsenic poisoning (Yorifuji et al., 2010). These data provide additional evidence that an early-life high-dose pulse arsenic exposure scenario can be associated with arsenic carcinogenesis in adulthood in humans (Yorifuji et al., 2010).

**ARSENIC AND STEM CELLS**

Stem cells (SCs) are crucial for normal organ and organismal development. The tightly regulated processes of self-renewal and differentiation maintain the delicate homeostatic balance between terminally differentiated cells and new SC activation in the organism throughout its entire lifetime. During the fetal period, SCs are necessary for normal tissue and organ generation and development. In the adult, SCs are critical for the maintenance of tissue self-renewal and organization and for tissue regeneration following stress or injury. Cancer SCs exhibit these same properties during tumor initiation and maintenance and during tumor regeneration following treatment (Bomken et al., 2010; Lobo et al., 2007; Pardal et al., 2003; Visvader and Lindeman, 2008). Many recent studies have shown that the signaling pathways involved in self-renewal and differentiation of normal SCs are often dysregulated in cancer cells (Bomken et al., 2010; Lobo et al., 2007; Pardal et al., 2003; Reya et al., 2001). Because of these shared properties and disrupted signaling pathways in CSCs, tumors can be viewed as an abnormal “organ” in which a small subpopulation of cells (i.e., CSCs) self-renew and differentiate but in a highly uncontrolled manner.

Arsenic compounds have been shown to affect SC dynamics and differentiation in various cell model systems and in vivo (Patterson and Rice 2007; Patterson et al., 2005; Tokar et al., 2010c,d; Waalkes et al., 2008). For example, arsenic exposure in vitro maintains epidermal cells in a germinative state, essentially blocking the progression of the putative SC/progenitor cells to a more differentiated phenotype (Patterson and Rice 2007; Patterson et al., 2005). Our group recently demonstrated that arsenic alters SC population dynamics during skin carcinogenesis, leading to dramatically increased numbers of CD34-positive skin CSCs in the resultant arsenic-induced
skin SCC (Waalkes et al., 2008). Chronic arsenic exposure in vitro can directly transform human prostate epithelial SCs into a pluripotent CSC phenotype (Tokar et al., 2010c). During this acquisition of malignant CSC phenotype, an early depletion and subsequent reactivation of SC self-renewal–related genes (p63, ABCG2, BMI-1, sonic hedgehog, OCT-4, and NOTCH-1) occurred. This alteration of self-renewal pathways is consistent with other studies (see Lobo et al., 2007; Pardal et al., 2003; Reya et al., 2001; Visvader and Lindeman, 2008), and the “U-shaped” expression trend in self-renewal genes is not specific to arsenic as a similar trend has been seen during the progression of other malignancies (Krivtsov et al., 2006). The reactivation of self-renewal genes and the acquisition of malignant phenotype occur concurrently with a progressive depletion of the phosphatase and tensin homolog (PTEN) tumor suppressor gene (Fig. 2; Tokar et al., 2010c). PTEN is important because its depletion enhances self-renewal capacity of SCs, increases tumorigenicity of stem-like cells, and leads to cancers enriched in CSCs (Dubrovská et al., 2009; Groszer et al., 2006; Yilmaz et al., 2006). PTEN depletion may be an important factor in arsenic carcinogenesis and arsenic-induced CSC formation, although further study is needed to fully confirm this notion.

The targeting of SCs by arsenic during malignant transformation may be due, at least in part, to an innate resistance and hyperadaptability of these cells to the metalloid in comparison with their mature counterparts (Tokar et al., 2010d).

FIG. 2. Diagram of prospective events that occur during arsenic-induced malignant transformation of normal human prostate epithelial SC/progenitor cells into cancer stem-like cells. Early events include a period of aberrant differentiation and depletion of SC self-renewal–related genes. A subsequent progressive depletion of the PTEN tumor suppressor gene causes a concurrent reactivation of self-renewal genes during the acquisition of malignant phenotype. These events give rise to CSCs that form highly pluripotent tumors when inoculated into nude mice (see Tokar et al., 2010c).

This resistance and hyperadaptability in SCs result in a marked CSC overabundance following arsenic-induced acquisition of malignant phenotype but was not seen in isogenic cadmium- or N-methyl-N-nitrosourea (MNU)-induced malignant transformants (Tokar et al., 2010d), suggesting that this phenomenon may be specific to arsenic. Similar results were seen with arsenic and cadmium in cultured human keratinocyte cells (Patterson et al., 2005). In vivo correlation of this concept comes with the markedly increased numbers of putative CSCs in skin cancers facilitated by in utero arsenic exposure compared with tumors produced without fetal arsenic exposure. Specifically, the arsenic-facilitated SCC of the skin clearly showed much higher levels of CSCs (Waalkes et al., 2008).

Cancers are thought by many to arise because of a maturation arrest during the progression of SC to transient committed progeny to differentiated cell (Sell, 2006). It is hypothesized, as in the case of chronic tissue repair, that aberrant alterations in self-renewal pathways may prevent activated SCs from returning to a quiescent state that usually follows regeneration, thereby enhancing the probability of an oncogenic event during chronic injury (Beachy et al., 2004). Arsenic appears to have the capacity in vitro to trap cells in the SC-like phenotype (Patterson et al., 2005) and also appears to target this phenotype for malignant transformation forcing an overabundance of CSCs with acquisition of malignant phenotype (Tokar et al., 2010c,d). The depletion and reactivation of self-renewal genes and concurrent depletion of PTEN in chronic arsenic-exposed SCs (Tokar et al., 2010c) could, in part, be responsible for preventing the return to quiescence and keeping the SCs in an activated state of self-renewal. This trapping of SCs in a state of self-renewal would be a highly effective mechanism to increase the pool size of target cells for arsenic and, thereby, greatly enhance the probability of an oncogenic event. It would also suggest that arsenic exposure during times when SC numbers are normally at higher levels and generally more active, such as during the fetal and/or early developmental life stages, would increase the risk for arsenic-induced cancer formation. This would help to explain why arsenic-induced cancers occur in adult mice exposed to arsenic during the fetal life stages (Tokar et al., 2010b; Waalkes et al., 2003, 2004b, 2006a,b) but do not generally occur in rodents exposed only during the adult stage of life (Tokar et al., 2010a), the time when SCs are at their lowest levels in most tissues.

SUMMARY

There is rapidly accumulating human evidence that fetal and/or developmental inorganic arsenic exposure is associated with cancer in adulthood (Liaw et al., 2008; Marshall et al., 2007; Smith et al., 2006; Yorifuji et al., 2010; Yuan et al., 2010). This includes data from environmental exposures via the drinking water (Liaw et al., 2008; Marshall et al., 2007; Smith et al., 2006; Yuan et al., 2010) and from poisonings because of
accidental arsenic food contamination (Yorifuji et al., 2010). The mouse transplacental model has similarly shown a remarkable ability to produce tumors in adult offspring after exposure to inorganic arsenic via the maternal drinking water during gestation (Tokar et al., 2010b; Waalkes et al., 2003, 2004b, 2006a,b). With additional treatments after fetal arsenic exposure, tumor response can be exaggerated or tumors at additional sites can be produced (Tokar et al., 2010b; Waalkes et al., 2004b, 2006a,b, 2008). Together, these human and rodent data provide a solid basis for declaring this as an instance of toxicant-induced developmental basis of adult disease. It appears for both in vivo and in vitro work that SCs may play a key role in this arsenic-based response, at least in some instances (Patterson and Rice 2007; Patterson et al., 2005; Tokar et al., 2010c,d; Waalkes et al., 2008). Additional effort should be directed into defining the role that fetal SCs play in how developmental arsenic exposure causes cancer in adulthood.

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