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Pesticides and oncogenic modulation

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ABSTRACT

Pesticides constitute a diverse class of chemicals used for the protection of agricultural products. Several lines of evidence demonstrate that organochlorine and organophosphate pesticides can cause malignant transformation of cells in *in vitro* and *in vivo* models. In the current minireview a comprehensive summary of recent *in vitro* findings is presented along with data reported from human population studies, regarding the impact of pesticide exposure on activation or dysregulation of oncogenes and tumor suppressor genes. Substantial mechanistic work suggests that pesticides are capable of inducing mutations in oncogenes and increase their transcriptional expression *in vitro*, whereas human population studies indicate associations between pesticide exposure levels and mutation occurrence in cancer-related genes. Further work is required to fully explore the exact mechanisms by which pesticide exposure affects the integrity and normal function of oncogenes and tumor suppressor genes in human populations.

1. Introduction

Cancer is a disease that occurs due to uncontrolled cellular proliferation. Initially it was believed that cancer originates through non-oxidative metabolic pathways that differ to the classical Krebs cycle. In the late 70s Varmus and Bishop discovered that cancer arises from specific genes that have undergone mutations and possess the ability to transform normal cells to cancerous cells. The first gene discovered that encompasses this ability was the src gene, that was named under the Rous Sarcoma virus. The latter could trigger neoplasms in chickens. Further research over the last years has increased our understanding of how cancer develops. Ultimately cancer is a disease that is based on two specific classes of genes: (1) the oncogenes and (2) the tumor suppressor genes. Oncogenes are genes that control mainly cellular proliferation and are either mutated or expressed at higher levels than normal in cancer cells. Tumor suppressor genes are genes that protect the integrity of the genome by inhibiting the cell cycle when substantial errors or mutations have occurred and are usually downregulated or inactivated in cancer cells. Oncogenes encode proteins that are involved in signal transduction from the extracellular environment and the cytoplasmic region toward the nucleus, where transcription is initiated. These proteins include growth factor receptors, cytoplasmic proteins involved in signal transduction and cellular proliferation and transcription factors regulating the transcription of certain genes. Tumor suppressor genes encode for proteins mainly involved in cell cycle arrest or apoptosis that are either cytoplasmic or nuclear transcription factors.

Mutations in the coding regions of oncogenes and tumor suppressors occur as a result of genetic and environmental causes. For example the transformation of the src proto-oncogene to its mutated oncogenic form was first characterized as a result of viral transfer, during which the initial sequence of the proto-oncogene was altered. However the vast majority of mutations originate from the hazardous effect of chemical substances present in the environment upon the genome. Several compounds have been established as carcinogens, according to the IARC (International Agency for Research on Cancer) criteria, which means that they are capable of inducing malignant transformation of normal cells in animals and humans. Generally there are four groups that define the carcinogenicity of a chemical: group 1 refers to compounds that are definitely carcinogenic to humans, group 2 and 3 refer to compounds that are probably and possibly carcinogenic to humans respectively, whereas group 4 includes compounds that are probably not carcinogenic to humans. With respect to toxicity caused to DNA, there are two classes of carcinogens, genotoxic and non-genotoxic carcinogens, *i.e.* compounds that can cause genetic damage or mutations by binding to DNA or carcinogenic compounds or agents that do not directly interfere with the genetic material of the cells. The carcinogenesis process includes 3 stages initiation, promotion and transformation.

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Pesticides constitute a diverse class of xenobiotics encountered frequently in the environment that are extensively used for the protection of crops and for increasing the yield of agricultural products. Pesticides are divided into various classes notably organophosphates, organochlorines, carbamates and pyrethroids. Organophosphate pesticides are based on the common chemical structure of phosphoric acid esters. Organochlorine pesticides are chlorinated hydrocarbons with no generic formula; however most of them are composed of 5 or 6 membered carbon rings that possess chlorine atom substitutions (DDT or chlordane). Carbamates are compounds that are derived from carbamic acid (NH_2COOH) and pyrethroids are derivatives the natural compound pyrethrin that is produced in flowers. Exposure to pesticides occurs as a result of occupational (*e.g.* spraying fields) work as well as environmental factors such as contamination of drinking water and food and may elicit various symptoms concerning human health.

Associations of symptoms such as hepatitis, cardiovascular disease, prostate cancer and thyroid function with long term exposure to organophosphate and organochlorine pesticides have been reported by numerous studies (Tsatsakis et al., 2009, 2011; Lacasana et al., 2010). In this minireview, we will emphasize on the relation between pesticide exposure and dysregulated cancer-related genes. Evidence from *in vitro* mechanistic and clinical exposure studies will be summarized with particular focus on the interactions of pesticides upon oncogenic proteins and tumor suppressor proteins.

2. Organochlorines and oncogenes

Most studies in the literature have focused in the carcinogenicity caused by organochlorine pesticides in animal and *in vitro* models. Several organochlorine pesticides are suspected to cause cancer in humans. For example the chlorotriazine terbuthylazine has been shown to induce DNA damage in human lymphocyte cultures, as determined by comet assay, as well as impair the structural integrity of c-myc and TP53 genes as a result of prolonged exposure (Mladinic et al., 2012). The pesticide beta-hexachlorocyclohexane (β-HCH), a contaminant of the pesticide lindane increases the mRNA expression of MMP-13, a marker of invasiveness *in vitro*, and the expression of a number of proto-oncogenes notably c-Neu, cyclin D1 and p27 (Wong and Matsumura, 2007). *In vivo* β-HCH accelerates the appearance of mammary tumors in the MMTV-Neu mouse model (Wong and Matsumura, 2007), thus indicating its highly carcinogenic potential. The structurally similar compound hexachlorobenzene, an environmental pollutant, is known to cause liver tumors in animals through a mechanism involving activation of c-myc, c-fos, c-jun proto-oncogenic proteins and PKC activity induction (Randi et al., 2003). A similar finding has been obtained for the chemical pollutant dichlorobenzene where changes in the expression of c-myc and Ha-ras oncogenes were noted in F344 rats, following administration of the compound (Kulkarni et al., 1999). The organochlorine herbicide 2,4-dichlorophenoxy acetic acid (2,4-D) induces cell transformation and increases the expression of c-myc transcription factor to the mRNA and protein level in syrian hamster embryo cells (Maire et al., 2007). In addition 2,4-D induced apoptosis in the above mentioned model as a result of DNA fragmentation (Maire et al., 2007). Therefore it has been proven that pesticides activate oncogenes *in vitro* and *in vivo*. Some organochlorine pesticides such as lindane have been banned during the last decade due to their highly carcinogenic action. In addition lindane is reported to disrupt macroautophagy to the molecular level by promoting vaquolation of Sertoli cells and that this defect is independent of mTOR and p38 pathways (Corcelle et al., 2006). In contrast the ERK pathway is a necessary requirement for lindane to disrupt the autophagic pathway (Corcelle et al., 2006).

Furthermore there is evidence indicating that the organochlorine atrazine enhances the carcinogenic effects of the polycyclic aromatic hydrocarbon (PAH) 7,12-dimethylbenz[a]anthracene. PAHs are environmental pollutants that are activated to carcinogenic reactive intermediates by phase I enzymes. Mice carrying copies of the human c-Ha-ras proto-oncogene were susceptible to increased incidence of mammary adenomas and adenocarcinomas caused by atrazine treatment, suggesting that endocrine disruptors such as atrazine may enhance mammary carcinogenesis in a certain limited dose (Fukamachi et al., 2004). Finally the organochlorine pesticide methoxychlor increases the rate of ovarian atresia in mice by increasing the percentage of atretic antral follicles *via* Bax upregulation (Borgeest et al., 2004). Fig. 1 outlines a putative scheme underlying possible mutagenic effects of pesticides on ongogenes and tumor suppressors.

3. Non-organochlorine pesticides and oncogenes

Evidence regarding the carcinogenic effect of other classes of pesticides is limited. The organophosphate pesticides parathion and malathion can induce malignant transformation of the normal epithelial breast cell line MCF-10F, as shown by anchorage independent growth capability and invasive characteristics (Calaf et al., 2009). The latter compounds caused an increase in p53 and c-Ha-ras protein expression, along with microsatellite instability for p53 at loci 17p13.1 and for c-Ha-ras at loci 11p14.1 (Calaf et al., 2009). Similarly paraoxon, the ester of the organophosphate parathion induces expression of c-fos gene in lymphocytes and consequently activation of N-terminus acetylcholinesterase expression (N-AchE) (Charoenying et al., 2011). C-fos induction has further been reported to occur by permethrin, a type I pyrethroid pesticide in primary cultures of mouse cerebellar granule cells, accompanied by activation of brain-derived neurotrophic factor gene and Ca^{++} influx into neurons, suggesting that pyrethroids can alter activity-dependent gene expression in neurons (Imamura et al., 2000). Finally carbofuran, a carbamate pesticide causes migration of DNA into the tail and impaired structural integrity of c-myc and TP53 genes in lymphocyte cultures (Mladinic et al., 2012). Collectively the effect of non-organochlorine pesticides upon oncogenic activation in isolated cultures *in vitro* is apparent, however the exact impact of this process in populations heavily exposed to pesticides remains unclear.

4. Pesticides and oncogenes: population studies

A few key studies have explored the frequency of mutations on oncogenes and tumor suppressors in populations occupationally and environmentally exposed to pesticides. The first and most conclusive study that examined the potential interactions of pesticide occupational exposure and increased risk of non-Hodgins lymphoma in north Central United States, in terms of G-banded chromosome analysis, was performed by Garry and colleagues (Garry et al., 1996). Significantly increased rearrangement frequencies were demonstrated in fumigant and insecticide appliers compared to control subjects, while chromosome bands contained oncogenes and genes involved in tumor suppression (Garry et al., 1996). The study undertaken by Slebos and colleagues in 2000 examined the presence of mutations at codon 12 of the ras gene in 61 patients with pancreatic cancer that were environmentally exposed to organochlorine pesticides (Slebos et al., 2000). The results were suggestive of the presence of K-ras codon mutations in patients with higher serum levels of DDEs (Slebos et al., 2000). Porta et al. in 1999 reported significant associations in a case control study of 51 subjects with exocrine pancreatic cancer between serum concentrations of p-p′-DDT and K-ras mutations in codon 12,

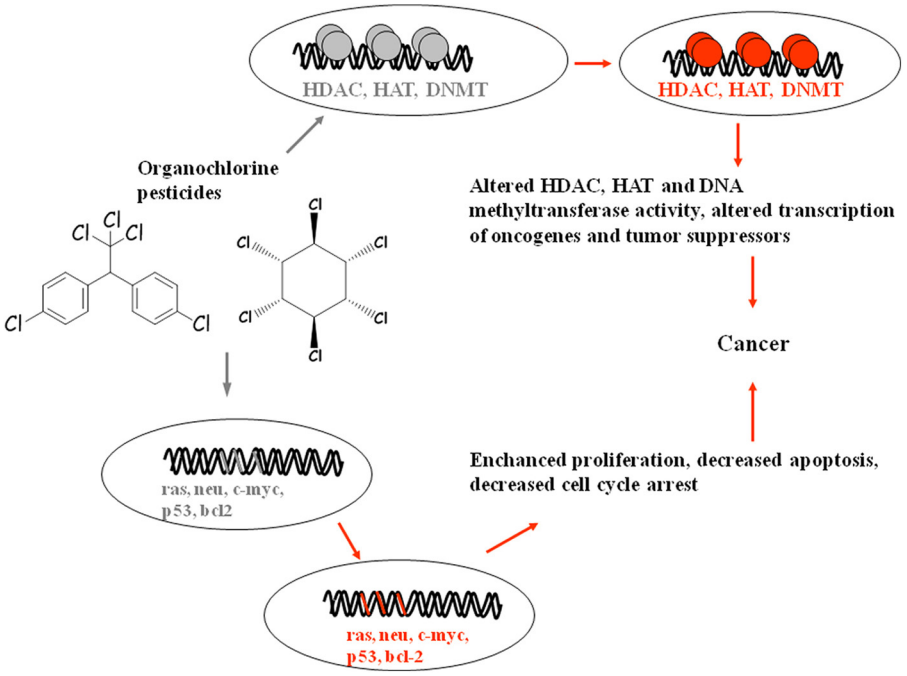

Fig. 1. The effect of organochlorine pesticides on the genome and the epigenome. Derivatives of lindane and methoxychlor can induce genetic alterations in the structural integrity of oncogenes and tumor suppressor genes. Red stripes indicate mutations occurring after pesticide exposure. In addition pesticides can promote enhanced or decreased expression of epigenetic enzymes such as Histone Deacetylases, Histone Acetyltransferases and DNA methyltransferases and in turn regulate the transcription of genes involved in cell cycle control and apoptosis. Red circles indicate altered epigenetic regulation of oncogenes and tumor suppressor genes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

reinforcing the findings by Slebos and colleagues (Porta et al., 1999). Thus organochlorine compounds such as p-p′-DDT and p-p′-DDE could play a part in the pathogenesis of exocrine pancreatic cancer *via* modulation of K-ras activation, although the exact mechanism in human populations remains unclear (Porta et al., 1999). A more recent study by Roulland et al. in 2004 examined the incidence of non-Hodgins lymphoma, in terms of BCL2-IGH translocation, in 56 individuals occupationally exposed to pesticides in open field farming (Roulland et al., 2004). The results suggested that occupational exposure to pesticides would increase the BCL2-IGH-bearing cells especially during the high pesticide use period, thus proposing the use of BCL2-IGH translocation measurements as a measure of acquired genetic instability, in relation to genotoxic exposure (Roulland et al., 2004). Based on the above studies it can be concluded that occupational risk of pesticide exposure is an important determinant of mutation development at key-genes involved in cellular proliferation and cell cycle control. The exact mechanisms that underlie the progression of a "healthy" to an "oncogenic" genotype in human populations exposed to pesticides remain unclear, mainly due to the plethora of chemical used, the main route and severity of exposure, as well as the complexity of genomic information of each population.

5. Pesticides and the "epigenome"

In addition to the damaging effect of pesticides upon the genome, major focus has been paid recently to the interaction of environmental exposure to pesticides with epigenetic elements. The "epigenome" as it has been characterized constitutes genomic information that has undergone pre- or post-transcriptional modification, such as methylation, acetylation and control of mRNA transcriptional activity. Organochlorine pesticides such as dieldrin and paraquat induce histone H3 acetylation and decrease HDAC (Histone Deacetylase enzyme) activity (Song et al., 2010, 2011). This results in higher levels of gene transcription, as the positively charged ions of histones are removed, thus decreasing the interaction of histones with the negatively charged phosphate groups of DNA. Similarly several pesticides have been reported to induce DNA methylation *in vitro* (Ray and Richards, 2001) that is an important factor for the epigenetic regulation of genes involved in diseases. Genome hypomethylation has been found in tumors (Das and Singal, 2004), whereas a recent study reported a strong correlation between increasing levels of persistent organic pollutants (POPs), such as the banned pesticide DDT, and global DNA hypomethylation (an aberrant epigenetic pattern of malignant cells) in a sample size of 70 subjects in the Greenlandic Innuit (Rusiechi et al., 2008). A general outline of these effects is shown in Fig. 1. Finally some pesticides have been shown to affect the expression of oncogenic miRNAs *in vitro* that are important molecules involved in the control of mRNA gene transcription. However this research area is a relatively new field and additional input in terms of experimental findings is required in order to fully understand the precise role of miRNAs in cancer and the effects of pesticides upon miRNA regulation.

Conflict of interest statement

There is no conflict of interest to declare with respect to this article.

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