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Review

REVIEW

Assessing the carcinogenic potential of low-dose exposures to chemical mixtures in the environment: focus on the cancer hallmark of tumor angiogenesis

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Abstract

One of the important 'hallmarks' of cancer is angiogenesis, which is the process of formation of new blood vessels that are necessary for tumor expansion, invasion and metastasis. Under normal physiological conditions, angiogenesis is well balanced and controlled by endogenous proangiogenic factors and antiangiogenic factors. However, factors produced

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by cancer cells, cancer stem cells and other cell types in the tumor stroma can disrupt the balance so that the tumor microenvironment favors tumor angiogenesis. These factors include vascular endothelial growth factor, endothelial tissue factor and other membrane bound receptors that mediate multiple intracellular signaling pathways that contribute to tumor angiogenesis. Though environmental exposures to certain chemicals have been found to initiate and promote tumor development, the role of these exposures (particularly to low doses of multiple substances), is largely unknown in relation to tumor angiogenesis. This review summarizes the evidence of the role of environmental chemical bioactivity and exposure in tumor angiogenesis and carcinogenesis. We identify a number of ubiquitous (prototypical) chemicals with disruptive potential that may warrant further investigation given their selectivity for high-throughput screening assay targets associated with proangiogenic pathways. We also consider the cross-hallmark relationships of a number of important angiogenic pathway targets with other cancer hallmarks and we make recommendations for future research. Understanding of the role of low-dose exposure of chemicals with disruptive potential could help us refine our approach to cancer risk assessment, and may ultimately aid in preventing cancer by reducing or eliminating exposures to synergistic mixtures of chemicals with carcinogenic potential.

Abbreviations

AHR	aryl-hydrocarbon receptor
CXCL9 and 10	C-X-C motif chemokine ligands 9 and 10
CCL2	monocyte-like chemoattractant protein
COLIII	collagen III
ECM	extracellular matrix
FGF	fibroblast growth factor
HIF-1α	hypoxia-inducible factor 1 alpha
HUVEC	human umbilical vein endothelial cells
HPTE	2,2-bis-(p-hydroxyphenyl)-1,1,1-trichloroethane
HTS	high-throughput screening
IL	interleukin
ICAM1	intercellular adhesion molecule 1
MMP1	matrix metalloproteinase-1
PAR	protease-activated receptors
PFOS	perfluorooctane sulfonate
THBD	thrombomodulin
TF	tissue factor
TGF-β	transforming growth factor-beta
uPAR	urokinase-type plasminogen activator receptor
VCAM1	vascular cell-adhesion molecule 1
VEGF/VEGFR	vascular endothelial growth factor/receptor
VEC	vascular endothelial cells

Introduction

Angiogenesis, the formation of new blood vessels from existing blood vessels, was identified as one of the 'hallmarks of cancer' by Hanahan and Weinberg (1,2) due to the recognition that this process is of crucial importance during the transition from benign hyperplastic nodules to malignant lesions (3). This review article focused on angiogenesis constitutes an integral component of the 2013 Halifax Project on 'Assessing the Carcinogenic Potential of Low-Dose Exposures to Chemical Mixtures in the Environment' (see Capstone Article for details). Tumor expansion is dependent on the ability of the tumor to induce the growth of new blood vessels, which provide nutrients and oxygen to the growing tumor mass and simultaneously serve as a conduit for tumor cells to metastasize to distant organs (4,5). Tumor angiogenesis is integral not only in solid tumor progression but also in leukemia (6). Recent cancer treatments target tumor angiogenesis using antiangiogenesis inhibitors (7,8), which prevent new vessel formation, or by using vascular-disrupting/damaging agents (9–11) and neovascular-targeting immunoconjugates (12–14). However, angiogenesis is also necessary for normal organ function, tissue growth and regeneration (e.g. wound healing, female menstruation, ovulation and pregnancy), necessitating a fine balance to avoid complications due to antiangiogenic therapy (15–17).

Though human exposures to environmental chemicals, which often occur due to the leaching of plastics into food and water (18), have been found to promote tumorigenesis of multiple cancers through various mechanisms (19–24), less attention has been focused on their role in tumor angiogenesis. With increases in our knowledge of endocrine disruptors (25), new concerns have arisen about potential exposures to low doses of environmental chemicals that are generally regarded as non-carcinogens, but may be acting as proangiogenic agents. Here, we consider the possibility that certain chemical disruptors, which are prevalent in the environment (e.g. as pesticides and industrial surfactants) (26), may have a role to play in environmental carcinogenesis by stimulating proangiogenic pathways, providing an environment conducive to tumor growth and metastasis.

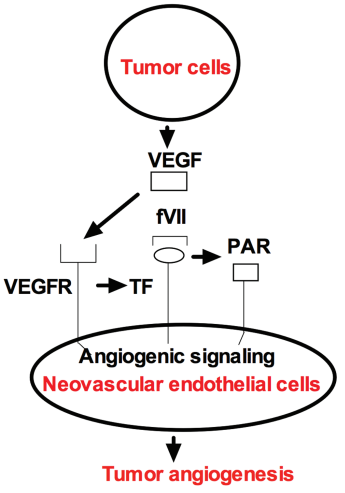
In this review, we discuss emerging data on specific environmental chemicals that may act as proangiogenic agents, and identify key angiogenesis pathways and corresponding molecular components as prioritized targets for future study. We briefly summarize *in vitro* and *in vivo* angiogenesis model systems with an emphasis on high-throughput screening (HTS) assays. We also consider the cross-hallmark relationships that a number of important angiogenic pathway targets have with other hallmarks of the disease and we make recommendations for future research.

Identifying VEGFR- and TF-mediated signaling as two key tumor angiogenesis pathways and corresponding molecular components as prioritized targets for assessing the carcinogenic potential of low-dose exposures to chemical mixtures in the environment

Tumor growth and metastasis require angiogenesis to provide a circuit for increased blood supply and dissemination of tumor cells (27). Angiogenesis is tightly controlled by diverse subsets of ligands and receptors. Enrichment of ligands, including growth factors, chemokines and cytokines or a decrease in the production of endogenous angiogenesis inhibitors, has been extensively observed in tumors during vascularization. The biology and mechanisms of tumor angiogenesis have been elegantly summarized elsewhere (4,28–33). Here, we will only briefly review some of the key angiogenic pathways [vascular endothelial growth receptor (VEGFR) and tissue factor (TF)-mediated signaling] (Figure 1A) and pathway-associated molecular components (Figure 1B) to provide a framework for our review and discussion of potential chemical disruptors (Figure 1B).

The vascular endothelial growth factor (VEGF) pathway is crucial for cancer angiogenesis. As a tumor enlarges, the tissue becomes hypoxic and deprived of nutrients leading to

A. VEGF/VEGFR and fVII/TF as two key angiogenic pathways



B. Potential angiogenic targets and corresponding chemical disruptors

Angiogenic targets	Proposed chemical disruptors
VCAM1	Diniconazole
CXCL9	Ziram
THBD	Chlorothalonil
CCL2	Biphenyl
ICAM1	Tributyltin chloride
uPAR	Methylene bis(thiocyanate)
COLIII	HPTE
CXCL10	PFOS
MMP1	Bisphenol AF
AHR	C.I. solvent yellow 14

Figure 1. VEGF and TF-signaling pathways as prioritized tumor angiogenic pathways (**A**) and proposed angiogenic molecular targets and their corresponding chemical disruptors (**B**). (A) The diagram shows VEGF produced by tumor cells binds to VEGFR on vascular endothelial cells to activate VEGF signaling pathways in tumor angiogenesis. In addition, VEGF binding to endothelial cells can induce TF expression, an angiogenic endothelial receptor in pathological neovasculature. After its ligand fVII binds, TF could contribute to tumor angiogenesis via proteolysis-dependent pathways through PARs or proteolysis-independent pathway through its cytoplasmic domain. (B) Proposed list of specific angiogenesis molecular targets and corresponding chemical disruptors.

increased expression of factors involved in both fighting against and adapting to these stressful conditions (34). Such factors will activate the growth of new blood vessels to increase the oxygen and nutrients supply but also lower the oxygen-dependent metabolism by causing a shift to glycolytic metabolism in the tumor cells (35). A well-studied example of hypoxia-induced tumor angiogenesis is the stabilization of hypoxia-inducible factor 1 alpha (HIF-1α) in hypoxic tumor tissues which lead to production of VEGF-A and nitric oxide synthase (NOS) that act as drivers of neovascularization and dilation of the existing blood vessels, respectively (36). In addition to VEGF-A, other growth factors including angiopoeitin-2 (Ang-2), fibroblast growth factors (FGFs), platelet-derived growth factors, insulin-like growth factor and transforming growth factor-beta (TGF-β) are also produced at high levels by hypoxic tumor or tumor stromal cells and lead to disruption of the tumor vessels (37). The tumor milieu, which has been compared to that of a healing wound (38), also leads to massive recruitment and activation of inflammatory cells types, including macrophages, neutrophils and lymphocytes, which are producing proangiogenic cytokines including tumor necrosis factor-alpha, interleukin 1 beta (IL-1β) and interleukin 6 (IL-6). In addition, carcinoma-associated fibroblasts are also rich sources of a wide range of angiogenic factors, further complicating the proangiogenic phenotype of solid tumors (39,40). In addition to angiogenic factors, deregulated vessel sprouting and path finding through disruptions in, for instance Notch-activation by delta-like ligand 4 (Dll4) and Jagged1 ligands (41), are involved in disrupting the tumor vascular functions further contributing to the pathological phenotypes of the tumor blood vessels. Activated endothelial cells and tumor-associated macrophages produce matrix metalloproteinases (MMPs) including a disintegrin and metalloproteases, MMP-2 and MMP-9, which cleave extracellular matrix (ECM) to release more ECM-bound angiogenic factors and further reduce the integrity of the vasculature, leading to a vicious circle driving pathologic progression in cancer (42,43). In addition to expressed proteins, angiogenesis-modulating miRNAs, so called angiomiRs, directly repress gene expression of several angiogenic or antiangiogenic factors by binding to the 3′-untranslated regions (3′-UTR) of target mRNAs (44). For instance, miR-21 promotes cancer progression and angiogenesis through Akt and ERK pathways (45).

As a consequence of such untamed and exaggerated angiogenic signaling by the broad palette of proangiogenic factors existing at high levels in the tumor, the vasculature become highly chaotic, immature and of very low quality (in terms of the stability and barrier function of the vascular wall) and functionality (in terms of supporting efficient perfusion through the tumor) (46). As such, tumor blood vessels exhibit excessive leakage, causing highly elevated interstitial fluid pressure and inhibited delivery of blood, paradoxically further contributing to tumor hypoxia and decreasing delivery of drugs injected to the blood stream. At the same time, such deregulated tumor blood vessels pose little opposition against tumor-cell intravasation and metastatic dissemination. As such the pathological vasculature can be considered a main cause of resistance to therapy and progression of the cancer to metastatic disease (47).

While tumor angiogenic vascular endothelial cells (VECs) may express VEGFR at higher levels (48), VEGFRs are not specific for angiogenic endothelial cells, but are constitutively expressed also in the quiescent vasculature in normal organs (49,50). In contrast, TF may be a promising target, which is specifically expressed by angiogenic vessels, making it more specific for the tumor vasculature than VEGF receptors. Under physiological conditions, TF is only expressed on some cells outside of vessels, but is not expressed by quiescent endothelial cells of blood vessels in normal organs (51). Accumulating evidence suggests that TF also contribute directly and indirectly to tumor angiogenesis (52–56). TF is a transmembrane protein receptor (57–60), which is composed of 263 amino acid (aa) residues with an extracellular domain (1–219 aa), a transmembrane domain (220–242 aa) and a short cytoplasmic domain (243–263 aa). As a type I membrane bound receptor, TF forms an exceptionally strong and specific complex with its natural ligand coagulation factor VII (61,62), the initial step of the coagulation

pathway (63). In tumor angiogenesis, it is found that TF expression is only detected on angiogenic tumor VECs (13,64–66), a downstream product induced by VEGF that can be secreted by cancer cells (67,68) and cancer stem cells (69). More importantly, TF is selectively expressed *in vivo* in the tumor neovasculature (12,13,64,65,70) and *in vitro* by VEGF-stimulated VECs, thus the latter could serve as an *in vitro* model of angiogenic endothelial cells (71–73). Other angiogenic factors and inflammatory chemokines (such as bFGF, IL-1β, tumor necrosis factor-alpha, bacterial lipopolysaccharide (LPS)) can also induce TF expression on VECs under pathological conditions (54). Thus TF can be regarded as an angiogenic-specific endothelial receptor (65,72,73). We believe that this unique feature makes TF a promising therapeutic target for neovascular-targeted therapy (73) and an interesting angiogenic receptor for discussion in this review and for future studies of chemical angiogenesis.

After induction by VEGF and other factors, vascular endothelial TF contributes to tumor angiogenesis via proteolysis-dependent and -independent signaling pathways (Figure 1A). More details on TF signaling in tumor angiogenesis were previously described and reviewed (52,74–77). Briefly, coagulation factor VII/TF complex can initiate the proteolysis-dependent pathway by activating protease-activated receptors (PARs), which is modulated by thrombomodulin (THBD), the endothelial-specific type I membrane receptor that binds thrombin to reduce thrombin generation, and ultimately results in the transcription of genes such as early growth response-1, adhesion molecules [intercellular adhesion molecule 1 (ICAM1), vascular cell-adhesion molecule 1 (VCAM1), P- and E-selectin], growth factors and cytokines (IL-6, IL-8, chemokines), whereas the cytoplasmic serine residues can be phosphorylated and ultimately influences endothelial cell migration. Note that many of these angiogenic components involved in VEGFR- and TF-mediated signaling are chosen as potential angiogenic targets for selected chemical disruptors (Figure 1B).

To review the role of low-dose exposures to environmental chemical disruptors in tumor angiogenesis, our angiogenesis team as part of the Halifax Project was asked to identify 10 angiogenesis molecular targets and 10 corresponding potential chemical disruptors for these angiogenic targets. We choose the following angiogenic components involved in VEGFR- and TF-signaling pathways as prioritized VCAM1, C-X-C motif chemokine ligands 9 and 10 (CXCL9 and CXCL10), THBD, monocyte-like chemoattractant protein (CCL2), ICAM1, urokinase-type plasminogen activator receptor (uPAR), collagen III, MMP1 and aryl-hydrocarbon receptor (AHR). These targets were chosen based on their relevance to the signaling pathways reviewed above, and, importantly, based on previous work that examined a large database of animal toxicity studies (ToxRefDB; http://actor.epa.gov/toxrefdb/) and the concordance between tumor incidence *in vivo* and chemical activity profiles *in vitro*. The 10 molecular targets in Figure 1B were angiogenic signaling molecules that showed statistically significant associations with mammalian carcinogenicity (78).

This list of target sites was not intended to be comprehensive. Other targets exist, including well-known molecules such as collagen IV, CXCL4, thrombospondin, MMP9, etc., But we selected these targets because each of them are actively involved in tumor angiogenesis and all of them have been shown to be of considerable importance. For example, suppression of the angiostatic molecules CXCL9 and CXCL10 and upregulation of the proangiogenic chemokine CCL2 would provide a local environment of proliferative and migratory signals to endothelial cells forming new vessels to feed a tumor (79,80). Decreased THBD expression was highly correlated with tumor invasiveness, metastasis and lower survival rates (81,82). CCL2 is complementary to angiogenesis, through p53 regulation of CCL2 gene expression (83,84). ICAM1 is also complementary to angiogenesis through NF-κB-independent role for p53 in ICAM1 regulation that may link p53 to ICAM1 function in various physiological and pathological settings (85). CXCL10 is complementary to angiogenesis through activation of p53 and p53-responsive genes. Over expression of IP10 upregulated p53 and resulted in altered expression of p53-responsive genes such as the p21Cip1, p27kip1, NF-κB, Bax and PUMA genes and the mitochondrial translocation of Bax (86). The AHR is complementary to angiogenesis through its role in cell cycle regulation. AHR modulates angiogenesis through a mechanism requiring VEGF activation in the endothelium and TGF-β inactivation in the stroma. Activation of AHR by its various ligands disrupts contact inhibition and induces cell proliferation depending on the tissue and cell type involved (87–93). THBD is contrary to angiogenesis due to over expression of p53 suppressed THBD expression (94,95). It is also complementary to genetic instability (96,97) and resistance to cell death (98). uPAR is contrary to angiogenesis (wild type p53 downregulates uPAR expression). P53 acts as an uPAR mRNA binding protein that downregulates uPAR mRNA stability and decreases cellular uPAR expression. Codepletion of Cathepsin B and uPAR reduced the expression of cyclin D1, cyclin D2, p-Rb and cyclin E while the expression of Cdk2 was unaffected. The MMP1 is contrary when cross validated with evasion of antigrowth signaling hallmark (99–102). Inactivation of Rb leads to increased expression of MMP1 and dysfunction of p53. p53 inhibits basal and UV-induced MMP-1 expression in human dermal fibroblasts and p53 dysfunction caused by XPC defects in lung cancers may enhance tumor metastasis via increased MMP1 expression (99–101,103).

To examine the role of these angiogenic pathways and prioritized targets in chemical angiogenesis, we also identify 10 corresponding chemical disruptors (Figure 1B) as novel environmental chemicals in tumor angiogenesis, which are discussed below in the Sections of 'Environmental Carcinogens Affecting Angiogenic Pathways' and 'Identifying Novel Environmental Chemical Disruptors'.

Environmental carcinogens affecting angiogenic pathways

Here, we review the evidence for proangiogenic actions of cigarette smoke, nicotine and arsenic as case study compounds that provide supporting evidence for the subsequent selection of environmental chemicals that disrupt angiogenic signaling targets and potentially contribute to cancer.

Cigarette smoke

Cigarette smoke is one of the oldest environmental exposures linked to cancer (104) and contains numerous carcinogenic compounds, such as nicotine and its derivatives, described elsewhere (105,106). Cigarette and second hand smoke have both been shown to induce or be associated with angiogenesis by a variety of mechanisms, although separating angiogenic effects from other carcinogenic activities is a challenge. Mouse models of chronic colitis were found to have dose-dependent increases in blood vessel formation and VEGF protein expression following exposure to CS (107). Tumor growth, capillary density, plasma VEGF levels and circulating endothelial progenitor cells were significantly increased in mice subcutaneously injected with Lewis lung cancer cells after a 17-day exposure to second

hand smoke compared to mice exposed to clean room air. These results were attenuated with mecamylamine, an inhibitor of nicotine cholinergic receptors (108).

A hospital-based case-control study consisting of 730 urothelial carcinoma cases, 470 bladder cancers, 260 upper urinary tract urothelial carcinomas and 850 age-matched controls found significant correlations between bladder and upper urinary tract urothelial carcinomas (UUTUC) and both cigarette smoking and arsenic exposure (109). The risk for both bladder cancer (6.6; 95% CI, 3.1–13.9) and UUTUC (9.9; 95% CI, 4–24.5) were increased with the presence of VEGF polymorphisms associated with increased cancer risk.

Nicotine

Nicotine, one of the main carcinogenic components of cigarettes, has been found to influence angiogenesis. Several *in vitro* studies have linked nicotine to proangiogenic effects in cancer. The ERK/COX-2 pathway was suggested to play a role in nicotine-induced VEGF expression in gastric cancer cells after VEGF levels were decreased by inhibitors of MEK (U0126) and COX-2 (SC-236) (110). Nicotine was further shown to influence angiogenesis in lung cancer (111). Nicotine significantly stimulated HIF-1α protein and VEGF expression in human non-small cell lung cancer (NSCLC) cells. Increased capillary and tubule formation was shown in human umbilical VECs (HUVECs) following treatment with conditioned medium containing nicotine. The possible mechanism of nicotine-induced VEGF expression was investigated with human dermal microvascular endothelial cells, which showed that the nicotine acetylcholine receptor was needed for pro-migratory effects of VEGF and bFGF in culture (111). In addition, cultured HUVECs were observed to have increased cell proliferation, migration and tube formation following exposure to nicotine at concentrations similar to those found in smokers (112).

Although *in vitro* studies provide some evidence that nicotine has proangiogenic properties, animal studies further bolster nicotine as a promoter of neovascularization, as well as provide possible biological mechanisms. An increase in lesion growth and lesion vascularity was seen in lung cancer and atherosclerosis mouse models following nicotine exposure (113). In addition, in a mouse model of hind-limb ischemia systemically administered nicotine (100 µg/ml in drinking water) resulted in an increase of capillary density in the hind limb from 0.38 to 0.71 (95% CI 0.55–1.01) capillaries/myocyte compared to control. Later it was shown that nude mice injected subcutaneously with HT-29 cells, a colon cancer cell line, exhibited significant increases in both blood vessels and microvessel densities after drinking water containing 200 µg/ml nicotine for 25 days. VEGF expression correlated with microvessel density. B1 and b2-selective antagonists reversed nicotine-induced tumor growth; suggesting b-adrenoceptors may be involved in nicotine-induced angiogenesis in colon cancer (114). The growth rate of breast, colon and lung cancer tumor cells implanted in a chorioallantoic membrane model exhibited significant increases following 1 week of exposure to nicotine (115). This study further showed that nicotinic receptor antagonists and integrin avb3 antagonists abrogated nicotine-mediated angiogenesis, suggesting molecular and cellular mechanisms of nicotine-mediated angiogenesis (116).

Arsenic

Another carcinogen that shows angiogenic properties is arsenic, an environmental contaminant that humans may be exposed to via environmental, medical and occupational sources (117). An *in vitro* study using HUVECs revealed that low concentrations (≤ 1 µM) of sodium arsenite increased cell growth and vascular tubular formation compared to higher concentrations (> 5 µM) that induced cytotoxicity and inhibited angiogenesis (117). Low concentrations of arsenic also induced transcript expression of VEGF and von Willebrand Factor, an early detector of endothelial activation, in tumor metastasis. Several subsequent *in vitro* studies focused on the proangiogenic properties of arsenic in human microvascular endothelial (HMVEC) cells. Klei *et al.* (118) investigated signaling interactions between arsenic and alcohols using non-cytotoxic concentrations of arsenite (1–5 mM) with or without the presence of 0.1% ethanol. Data in this study showed that both agents together, but not ethanol alone, increased phosphorylation of the regulator of vascular integrin signaling PYK2 and VEGF gene expression as well as endothelial tube formation (118). Another study revealed that the sphingsine-1-phosphate type 1 receptor is important for arsenic-stimulated signaling for angiogenic effects (119) and that heme oxygenase-1 plays a role in arsenic-induced angiogenesis (120). Moreover, environmentally relevant levels of arsenic were shown to promote angiogenesis, neovascularization and inflammatory cell infiltration in Matrigel plugs implanted in C57BL/6 mice following 5 weeks exposures (drinking water) to concentrations ranging from 5 to 500 ppb (121).

These examples from the literature on known carcinogens indicate that environmental exposures to cigarette smoke, nicotine and arsenic can result in the increase of angiogenic activity through several pathways. There is a diversity of techniques available for investigating angiogenic activity, though there are challenges to separating effects that are specific to angiogenic pathways from other hallmark pathways.

Other environmental chemicals with proangiogenic properties

In addition to cigarette smoke, nicotine and arsenic, other potentially carcinogenic compounds have been identified that induce proangiogenic effects. Whole diesel exhaust has been shown to enhance angiogenesis in mice with either subcutaneous scaffold implantation or hindlimb ischemia (122). Increased CD31 expression, vessel volume and VEGF and HIF-1 gene expression was observed in these models. Bisphenol A has been intensively studied over the past few years due to its detrimental effects on developmental processes and metabolic effects and has recently been shown to influence angiogenesis (123). Increased gene expression of VEGFR-2, VEGF-A, eNOS and Cx43 and production of nitric oxide was found after HUVECs were exposed to 1M bisphenol A for 6 h (123). Furthermore, manganese induced hypoxia-associated transcript expression of proangiogenic genes in mice (124) and both dioxin (125) and trimethyltin chloride (109) were found to influence angiogenesis and vascularization during early development in rat and zebra fish models.

Identifying novel environmental chemical disruptors

As discussed above, tumor angiogenesis is critical for carcinogenesis, and despite the evidence that several known carcinogens are targeting proangiogenic pathways the role of most environmental chemicals in tumor angiogenesis is largely unknown. In this project, we were tasked to identify 'prototypical' environmental chemicals with disruptive potential that met the following criteria: chemicals that are ubiquitous in the environment; chemicals that have been shown to disrupt specific mechanisms/pathways for angiogenesis; and chemical exposures that are not related to 'lifestyle' choices (i.e. chemicals that are not already known or designated to be human carcinogens).

Our intent was to explore the possible synergies of disruptive environmental chemicals with proangiogenic capabilities that could potentially contribute to carcinogenesis (especially when combined, or when acting with other chemicals that are known to perturb other cancer hallmark pathways).

Thousands of untested chemicals in the environment lack hazard characterization of their carcinogenic potential. The Tox21 partnership of regulatory and scientific federal agencies, including USA. EPA, the National Toxicology Program (NTP), the National Center for Advancing Translational Science (NCATS) and USA. FDA, are addressing this data gap using *in vitro* HTS and computational modeling to predict hazard and prioritize compounds for targeted testing (126,127). The EPA's ToxCast research project (127), part of Tox21, has tested over a thousand chemicals with known and unknown toxicities in hundreds of assays for human gene and protein targets in pathways linked to cancer disease processes (128). This effort is concurrent with the creation of the Toxicity Reference Database (ToxRefDB) containing >40 years' worth of *in vivo* animal toxicity data, such as 2-year chronic cancer studies, broken down into a computable and searchable ontology structure (129). A model was recently published that used the ToxCast Phase I HTS data to predict *in vivo* rodent carcinogenicity endpoints from ToxRefDB (78). This work employed an unsupervised statistical approach to identify significant correlations between *in vitro* assay activity and preneoplastic and neoplastic lesions in a variety of tissue types, across a training set of 232 compounds with both *in vitro* and *in vivo* data. The model was able to accurately predict carcinogenicity classifications from the EPA's Office of Pesticide Programs for an external test set of compounds, based solely on their *in vitro* HTS data. Interestingly, the majority of HTS assays that were strongly associated with particular types of rodent cancers were linked to genes, pathways and hallmark processes documented to be involved in tumor biology and cancer progression, including stimulation of angiogenesis.

Most of the chemical carcinogens in the model training set were food-use pesticides, meaning they are non-genotoxic and instead act as tumor promoters (130). In addition to broad activity across assays that were mapped to other hallmark processes (i.e. apoptosis, proliferative signaling, evading growth suppression, enabling replicative immortality, metastasis, avoiding immune destruction, tumor-promoting inflammation and deregulating cellular energetics) some of these compounds were linked to targets in angiogenic pathways (1,2). Thus, a subset of these chemicals may have the potential to act as tumor promoters primarily via induction of angiogenesis, based on specific patterns of bioactivity against *in vitro* targets associated with vascular development. Many of these targets were from enzyme-linked immunosorbent assay-based chemokine expression assays in human primary cell cocultures. Statistically significant associations were observed between pesticide exposures causing rodent liver, thyroid, spleen and kidney tumors and differential regulation of inflammatory chemokines as well as cellular adhesion molecules, and elements of the plasminogen activating system. Many of these targets, shown in Figure 1, belong to signaling pathways reviewed above. Therefore, the results from the *in vitro* screens of these mammalian carcinogens were in all cases consistent with a proangiogenic and thus a protumorigenic program.

Analysis of bioactivity patterns of over a thousand chemicals across hundreds of *in vitro* assays revealed that other carcinogens were preferentially affecting targets in chemokine signaling, vascular cellular adhesion molecules and ECM interactions controlling vascular growth factor release (78). These results strongly support the concept that at some point in cancer progression, the angiogenic switch is turned 'ON', facilitating tumor growth, and that carcinogenic environmental chemicals may participate in this process by regulating cellular signaling in a proangiogenic fashion.

A number of environmental chemicals tested in the ToxCast program were identified as potential tumor promoters through their ability to interact with the angiogenic signaling molecules *in vitro* that had been shown to be significantly associated with *in vivo* tumorigenesis. Many of these compounds had associated *in vivo* data and evidence in the literature confirming their carcinogenic effects (78), while others are candidates for further study. In the ToxCast Phase I study, there were 27 chemicals tested in the *in vitro* assays for which there was no corresponding *in vivo* guideline data or EPA carcinogenicity classification (examples shown in Table 1). As shown in Figure 1B, we identify several of these Phase I compounds that may be acting via proangiogenic mechanisms, their cancer hallmark score and the specific angiogenic targets affected. All of these compounds are present in the environment, are predicted to be selectively disruptive, are not 'lifestyle'-related, and not known to be 'Carcinogen to Humans' (i.e. IARC Group 1). The Toxicological Priority Index (130) (ToxPi, key shown in Figure 2) displays the activity of each chemical against the angiogenic *in vitro* assay targets that were previously identified as significantly associated with tumor endpoints *in vivo*. The size of the slice is determined by the potency of the compound in the assay, based on the half-maximal activity concentration (AC_{50}). The chosen angiogenic prototypical disruptors are Bisphenol AF, Methoxychlor, perfluorooctane sulfonate (PFOS), Diniconazole, Ziram, Chlorothalonil, Biphenyl, Tributyltin Chloride, 2,2-bis-(p-hydroxyphenyl)-1,1,1-trichloroethane (HPTE) and C.I. Solvent Yellow 14 (Figure 1B). For several of these compounds, there is literature evidence that supports their potential angiogenic activity. For example, Bisphenol AF may induce angiogenesis via inactivation of the p53 axis and underlying deregulation of proliferation kinetics and cell death in human epithelial cells, as well as through its effect on Estrogen Receptor (ERα) (131). Bisphenol AF also affected a number of vascular targets in the ToxCast assay portfolio, including uPAR, THBD and ICAM1, as well as downregulating the antiangiogenic chemokines CXCL9 and CXCL10. Methoxychlor (the parent compound to HPTE) was shown to induce increases in histological expression of angiogenic factors such as VEGF, VEGFR2 and ANG1 in rat pituitary and uterus (132). The angiogenic HTS targets of HPTE include CXCL10, CXCL9, MMP1, uPAR, THBD, ICAM1 and VCAM1. Exposure to PFOS induced actin filament remodeling and endothelial permeability changes as well as ROS production in human microvascular endothelial cells (133). PFOS could also overwhelm homeostasis of antioxidative systems, boost ROS generation, impact the mitochondria and affect protein expression of apoptotic regulators in endothelial cells (134). Diniconazole (a pesticide) is predicted to be carcinogenic and shown to target certain angiogenic molecules CXCL10, uPAR and VCAM1 *in vitro*. Ziram may induce angiogenesis through activation of mitogen-activated protein kinases (MAPK) and decreases cytolytic protein levels in human natural killer cells (135,136).

Phase II of the ToxCast program expanded the chemical library beyond pesticides to over a thousand compounds, many of which lack cancer data but appear to be targeting angiogenic signaling and may also be candidates for future examination. A number of organotin compounds, including tributyltin chloride, tributyltin methacrylate and triphenyltin hydroxide, caused a decrease in expression of THBD in vascular smooth muscle cells as well as other proangiogenic activity in the ToxCast assays. As in the case of dioxin, AHR ligands

may be potential tumor promoters via angiogenic pathways, and it has been hypothesized that AHR signaling may suppress VEGF-A expression by competing with HIF-1α for their common dimerization partner ARNT (137). Compounds such as C.I. solvent yellow 14, Benzo(b) fluoranthene and 7,12 dimethyl(benz) anthracene are active in the AHR assay in addition to multiple

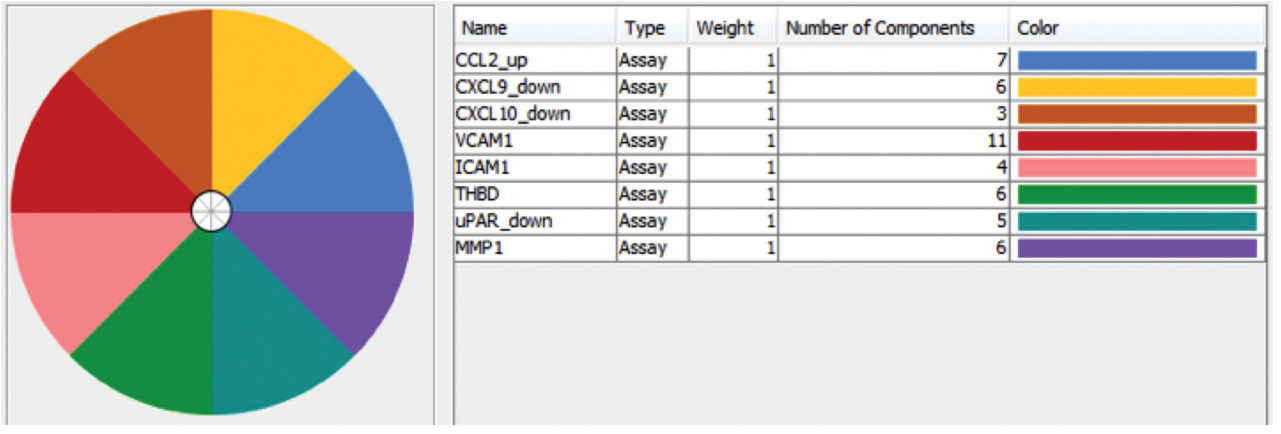
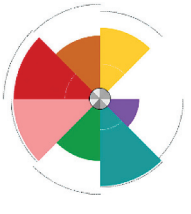


Figure 2. The ToxPi key for proangiogenic *in vitro* assay targets that were previously identified as being statistically significantly associated with tumor endpoints *in vitro*. The number of components represents the number of ToxCast assays for that target. Results for certain ToxCast Phase I test chemicals are shown in Figure 1B.

Table 1. Examples of ToxCast Phase I chemicals predicted to be carcinogens and shown to target certain angiogenic molecules *in vitro*, but lacking *in vivo* data or EPA carcinogenicity classifications

Chemical name	Chemical use class	Cancer hazard model score (#cancer hallmark assays hit)	Angiogenic targets	Proangiogenic ToxPi
Diniconazole	Pesticide	18	CXCL10, uPAR, VCAM1	
HPTE	Pesticide metabolite	17	CXCL10, CXCL9, MMP1, uPAR, ICAM1, THBD, VCAM1	
Methylene bis(thiocyanate)	Pesticide	16	CXCL10, CXCL9, MMP1, uPAR, ICAM1, THBD, VCAM1	
PFOS	Industrial surfactant	7	CXCL10, MMP1, uPAR, VCAM1	

These compounds were identified in an analysis linking rodent chemical carcinogenesis to HTS assay targets in cancer hallmark pathways (78). All of these compounds are ubiquitous in the environment, are predicted to be selectively disruptive, are not 'lifestyle' related and not known to be 'Carcinogen to Humans' (i.e. IARC Group 1). The Toxicological Priority Index (ToxPi) key mapping assays to slices is shown in Figure 2. CXCL9 and 10, C-X-C motif chemokine ligands 9 and 10.

other angiogenic targets, however their downstream effects on VEGF expression and angiogenesis will be dependent on their agonist vs. antagonist activity and are not yet known. Other chemicals exhibited specific activity on cytokine signaling, such as acrylamide and biphenyl, both of which caused increased expression of the proangiogenic chemokine CCL2 in vascular smooth muscle cells. The release of the full ToxCast Phase II dataset in late 2013 (http://www.epa.gov/ncct) is assisting in further identification of key assay targets and prioritization of potential chemical modulators of tumor angiogenesis. There are also a number of compounds that emerged from this analysis that have been tested in animals and assigned positive carcinogenicity classifications, but whose effects have not been well characterized histologically. If some of these were studied in more depth, they could also potentially serve as proangiogenic reference compounds.

In vitro and *in vivo* angiogenesis assays including HTS assay for assessing the effects of environmental chemicals in tumor angiogenesis

To screen the effects of environmental chemicals in tumor angiogenesis, there are many well developed *in vitro* and *in vivo* angiogenesis model systems that can be used or adapted (138–151). Each model has distinct advantages and disadvantages. Microvascular endothelial cells or well-characterized immortalized microvascular endothelial cell lines are generally considered superior to HUVEC in tumor angiogenesis studies, since tumor blood vessels are presumably microvessels. *In vitro* assays are usually designed to examine endothelial cell proliferation, migration and ability to form tube-like structures in coculture, matrigel or other matrix-containing environments. *In vivo* assays include, but are not limited to, the chorioallantoic membrane assay (chicken embryos), mesenteric window assay (small gut of rats and mice), corneal angiogenesis assay (rabbit, rat or mouse eyes), matrigel plug assay (mice and rats), sponge implant assay (rats) and alternate animal models such as hamster and zebrafish.

With technological advancement and the development of HTS, several *in vitro* angiogenesis assays have been used to screen and profile large numbers of chemical compounds that can be assayed in 96-well to 1536-well microplates. Because cancer cells can survive through compensation pathways, a battery of angiogenesis assays in HTS formats are needed to rapidly profile thousands of environmental chemicals and to build better predictive toxicology models. These assays are grouped into biochemical and cell-based categories and summarized in Table 2.

Biochemical HTS assays directly measure the effects of test chemicals on target protein or peptide samples. These methods are particularly useful for well-validated angiogenic signaling components. Several biochemical assays have been implemented in large scale screens for VEGFR (166), TF (171), TGF-β (175), HIF (176) and integrins (177). Particularly, Yauch *et al.* (171) described a HUVEC-based HTS assay for the VEGF signaling pathway followed by quantitative real-time PCR for measuring downstream gene products TF

Table 2. HTS assays for assessing the role of environmental chemicals in tumor angiogenesis

Assay technology	Target	Assay principle	HTS format	Reference
Biochemical HTS assays				
Fluorescence intensity	Integrin	Binding to dye-labeled fibronectin	Microarray	(163)
FP	VEGF,	Competitive binding of dye-labeled proteins or ligands	384 well, microfluidics	(164)
TRF	HIF-1α	Protein–ligand binding interactions	96 well	(176)
AlphaScreen	VEGFR	Protein–ligand binding interactions	1536 well	(165)
TR-FRET	TGF, VEGFR	Product formation catalyzed by active enzymes	96 well, 384 well	(175,166)
Cell-based HTS assays				
Phenotype	Tube formation	Total tube length measured by ds-Tomato fluorescent protein, nuclear stains or cell permeable dyes	96 well, 384 well, 1536 well, microfluidics	(179,167,168, 169, 170,172)
	Wound closures	Scratch assays or stopper assays, with some measured by cell permeable dyes	96 well, 384 well, microfluidics	(173,174, 357, 358,359)
Chemifluorescence	IL-1α/β, IL-6, IL-10	Detection of endogenous target proteins	96 well	(360)
β-lactamase reporter	IL-6, HIF-1α, NFκB,	Target-driven β-lactamase reporter gene system and β-lactamase-cleavable FRET substrates	384 well, 1536 well	(181,182,361,184)
GFP reporter	NFκB, VEGF, IL-8	Target-driven GFP reporter gene system	96 well, 384 well	(362–364)
Luciferase reporter	NFκB, HIF-1/2, VEGFR, IL-8, TGF- β	Target-driven luciferase reporter gene system	96 well, 384 well, 1536 well	(184,365,366,180, 185)
	HIF-1α	Degradation of a luciferase-fused HIF-1α reporter	384 well	(183)
TRF	E-selectin, ICAM-1, VCAM-1	Detection of endogenous targets	96 well	(367)
RT-PCR	VEGFR	mRNA levels of ICAM-1 and tissue factor	96 well	(171)

FP, fluorescence polarization; GFP, green fluorescent protein; HIF-1, HIF-2, HIF-1α, hypoxia-inducible factor 1, 2 and 1 alpha; ICAM-1, intracellular cell adhesion molecule 1; IL-1α, IL-1β, IL-6, IL-8 and IL-10, Interleukin 1 alpha, 1 beta, 6, 8 and 10; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; RT-PCR, real-time polymerase chain reaction; TRF, time-resolved fluorescence; TR-FRET, time resolved fluorescence resonance energy transfer.

Table 3. Cross-validation of angiogenic target pathways

Angiogenesis priority targets	Deregulated metabolism	Evasion of anti-growth signaling	Genetic instability	Immune system evasion	Resistance to cell death	Replicative immortality	Sustained proliferative signaling	Tissue invasion and metastasis	Tumor-promoting inflammation	Tumor microenvironment
VCAM1	+ (190)	0	0	0	+ (191)	0	+ (192)	+ (193–196)	+ (197)	+ (198)
CXCL9	0	0	0	– (199,200)	0	0	+ (201)	– (202–204)	+ (201,205,206)	+ (207)
THBD	0	– (94,95)	+ (96,97)	0	+ (98)	0	0	– (81,82,208,209)	– (209–211)	0
CCL2	+ (212)	+ (83,213)	– (214)	+ (215,216)	+ (217)	0	+ (218)	+ (219–221)	+ (222)	+ (223)
ICAM1	+ (190,224)	+ (85)	– (225)	– (226,227)	– (228)	0	+ (229)	+ (191,229–232)	+ (233,234)	+ (198,235)
uPAR	+ (236)	– (103,237–239)	0	0	+ (239,240)	+ (241)	+ (242)	+ (243–248)	+ (249)	– (250)
COLIII	+ (251)	0	0	0	0	0	0	+ (252–254)	+ (255)	0
CXCL10	0	+ (86)	0	– (199,256–259)	0	0	+ (260)	+/– (261–265)	+ (266,267)	+ (199,207)
MMP1	+ (268)	– (99–102)	+ (269)	0	0	0	+ (270)	+ (271–276)	+ (277)	+ (250,278)
AHR	+ (279)	+ (88–93,280)	+ (281)	0	+/– (87,282,283)	+ (284–286)	+ (287,288)	+/– (289)	+ (290)	+ (291)

Complementary effect (+): Targets and chemicals that were not only relevant for angiogenesis, but also relevant for other areas of cancer biology (i.e. procarcinogenic). Contrary effects (–): Targets and chemicals that were found to have opposing actions (i.e. anticarcinogenic). Both (+/–): Instances where reports on relevant actions in other aspects of cancer biology were mixed (i.e. reports showing both procarcinogenic potential and anticarcinogenic potential). Not known (0): Instances where no literature support was found to document the relevance of a target site or chemical in a particular aspect of cancer biology. VCAM1, vascular cell adhesion molecule 1; CXCL9 and 10, C-X-C motif chemokine ligands 9 and 10; CCL2, monocyte-like chemoattractant protein; ICAM1, intercellular adhesion molecule 1.

Table 4. Cross-validation of disruptors in other cancer hallmarks

Angiogenesis prototypical disruptors	Deregulated metabolism	Evasion of anti-growth signaling	Genetic instability	Immune system evasion	Resistance to cell death	Replicative immortality	Sustained proliferative signaling	Tissue invasion and metastasis	Tumor promoting inflammation	Tumor microenvironment
Diniconazole	+ (292)	0	0	0	0	0	0	0	+ (293)	0
Ziram	+/– (294,295)	+ (296)	+ (297)	0	– (298)	0	0	0	+ (298,299)	0
Chlorothalonil	+ (300,301)	+ (302)	+ (303)	0	– (304)	0	+ (305)	0	+ (306,307)	0
Biphenyl	0	Do not alter the levels of p53 (135)	+ (307)	0	– (308,309)	0	+/– (310,311)	– (312–314)	+ (315)	0
Tributyltin chloride	+ (316–319)	0	+ (320)	0	– (321–324)	0	0	0	+ (321,325)	0
Methylene bis(thiocyanate)	0	0	+ (326)	0	0	0	0	– (327–329)	+ (330)	0
HPTE	0	0	+ (331,332)	0	+ (333)	0	+ (334)	0	+ (335)	0
PFOS	0	+ (134,336)	+ (337)	0	– (338,339)	0	+ (340)	0	+ (341,342)	0
Bisphenol AF	+ (343,344)	+ (131)	0	0	– (345)	0	+ (346)	+ (347–349)	+ (350,351)	0
C.I. solvent yellow 14	+ (352,353)	+ (352,354)	+ (355)	0	0	0	0	0	+ (356)	0

Complementary effect (+): Targets and chemicals that were not only relevant for angiogenesis, but also relevant for other areas of cancer biology (i.e. procarcinogenic). Contrary effects (–): Targets and chemicals that that were found to have opposing actions (i.e. anticarcinogenic). Both (+/–): Instances where reports on relevant actions in other aspects of cancer biology were mixed (i.e. reports showing both procarcinogenic potential and anticarcinogenic potential). Not known (0): Instances where no literature support was found to document the relevance of a target site or chemical in a particular aspect of cancer's biology. HPTE,2,2-bis-(p-hydroxyphenyl)-1,1,1-trichloroethane; PFOS, perfluorooctane sulfonate.

and ICAM1 as transcriptional readouts. This HTS/real-time qPCR assay could be improved, e.g. using microvascular endothelial cells as discussed above, for future study of assessing chemical disruptors in tumor angiogenesis, as we propose in this review.

Cell-based HTS assays can be used to assess phenotypic changes or specific pathway activation/inhibition caused by exposure to test chemicals in cells or tissues. Active compounds identified from biochemical screens do not always exhibit similar activities in physiological conditions, thus cell-based assays, especially human primary cells, are useful to identify chemicals that exert adverse effects in the natural environment. Angiogenesis-associated phenotypic changes such as proliferation, apoptosis, motility and tube formation are routinely quantified in endothelial cells by a wide selection of commercially available assay kits and instruments (178,179). Chemicals that alter gene expression or protein–protein interaction can be detected by immunofluorescence or intracellular reporter gene assays. A battery of such assays have been applied to screen and identify chemicals targeting cellular signal pathways including HIFs (180,182,183,361), NF-κB (184), IL-6 (181), IL-8 (185) and TGFs (186,187).

The environmental chemicals can be assessed and profiled using the aforementioned assays in a quantitative HTS platform in which each test chemical is assayed at multiple concentrations covering at least four-log concentrations (188). The quantitative HTS-generated concentration response curves greatly reduce rates of false positives and false negatives, facilitating chemical prioritization for follow-up in-depth studies. For example, a cell-based hypoxia-response element-β-lactamase reporter assay has been optimized and miniaturized into a 1536-well format, and utilized to identify inhibitors and activators of the HIF-1 signaling pathway from 73 000 compounds from the Molecular Libraries Screening Centers Networks (MLSCN) (361) and 1408 environmental chemicals from the collection of the National Toxicology Program (182). Three environmental chemicals—iodochlorohydroxyquinoline, cobalt sulfate and *O*-phenanthroline were identified as chemical inducers of hypoxia signaling pathway. These quantitative HTS assays combined with a robotic system will greatly increase screening throughput for future assessment of environmental chemicals that may be affecting angiogenesis and other cancer hallmarks (189).

Discussion

When tumor vasculature was first successfully targeted in cancer to prevent growth and dispersion of malignant cells, it appeared that not only the blood vessels but the entire microenvironment within the tumor was participating in tumor growth, progression and resistance to treatment (152). A new concept providing additional relevant factors in this already complex multifaceted pathology was emerging to explain why current therapies are not fully or only transiently efficient (153). It is not only that 'normal cells' could turn into 'conscripted or subverted cells' to establish a cancer but some other normal cells would be triggered by the mutant cancer cells to help them proliferate and survive. These include normal host cells such as endothelial cells, fibroblasts, monocytes/macrophages, mesenchymal cells and cells of hematopoietic origin, at sites distant from and local to the site at which malignant transformation occurs (154). In addition, host and cancer cell interactions are occurring within a network that governs and influences both cancer and host cell properties. This ECM is now recognized as a crucial regulator of cancer evolution (152). Thus, several cell types in a complex and dynamic non-cellular environment collaborate to stimulate angiogenesis. One would therefore predict that chemical mixtures potentially modifying the tumor environment would therefore affect angiogenesis for the benefit of the cancer cells. On the other hand, tumor angiogenesis is also closely tied to hypoxia and thus deregulated metabolism, tumor-promoting inflammation, accelerated tumor growth, invasion and metastasis.

The carcinogenicity of low-dose exposures to chemical mixtures in any given tissue will probably depend upon simultaneous activation of several important tumor promotion mechanisms and the disruption of several important defense mechanisms. The potential synergies of combinations of chemicals will ultimately be involved in several mechanisms of disruptive actions that are known to be relevant in cancer biology. We undertook a thorough cross validation activity to illustrate the importance of the prioritized target sites for disruption (i.e. across multiple aspects of cancer's biology) and to illustrate the extent to which the prototypical chemical disruptors that were identified disrupt other mechanisms that are also relevant to carcinogenesis. Since tumor angiogenesis is not only an early and central event in the development of a tumor, but also critical and essential for tumor growth, invasion and metastasis. In addition, it is closely tied to hypoxia and deregulated metabolism. Therefore, we cross validate their potential participation of these angiogenic targets in other cancer hallmarks (Table 3) and their potential effects of chemical disruptors of angiogenesis in other cancer hallmarks (Table 4).

When studying the role of chemical disruptors in tumor angiogenesis, it is also important to keep in mind that inflammation and angiogenesis are closely linked (126,155–157). Many of the angiogenic molecule targets that are selected as important targets in this review are also involved in inflammation pathways. However, the critical role of VEGFR and TF pathways in chemical angiogenesis can be examined *in vitro* with HTS systems where individual chemical disruptors can be added to the assay wells to explore their role in angiogenesis, followed by a variety of assay techniques as reviewed and summarized above and in Table 2 for measuring the changes of these angiogenic priority targets (CCL2, ICAM1, CXCL9, CXCL10, AHR, THBD, uPAR, MMP1, VCAM1 and collagen III) that we choose as potential targets for chemical disruptors (Bisphenol AF, Methoxychlor, PFOS, Diniconazole, Ziram, Chlorothalonil, Biphenyl, Tributyltin Chloride, HPTE and C.I. Solvent Yellow 14).

It is worth noting that many common drugs and some dietary compounds can prevent cancer by inhibiting tumor angiogenesis. For example, aspirin and metformin are two cases where epidemiological evidence indicates cancer prevention (158,159), and experimental evidence suggested that inhibition of angiogenesis plays a part in this role (160,161). As well, there is substantial experimental evidence for phytochemicals, in particular dietary phytochemicals, preventing angiogenesis (162). So simultaneous exposures to both antiangiogenic and proangiogenic substances may represent two competing forces that could influence the process of environmental carcinogenesis. However, it is beyond the scope of this review to simultaneously consider these antiangiogenic exposures as well. Primarily, we believe that proangiogenic environmental exposures have not been considered in detail elsewhere, so they are the focus of this review. However, we do recognize that the combined effects of these constituents with other chemicals warrant careful consideration.

Conclusions

In conclusion, we propose to study the role of environmental chemicals on angiogenesis, particularly at low doses of selective

chemical disruptors. We believe there is a great need for future research that explores the potentially carcinogenic synergies produced by low-dose exposures to a wide range of chemicals with disruptive potential. Those with proangiogenic potential may be non-carcinogenic, but combinations of those chemicals may warrant further research and how they might combine with other chemicals that act on other hallmarks may help us better understand whether or not these types of combination exposures have a role to play in environmental carcinogenesis. In this regard, we identify prioritized vascular signaling targets, identify various environmental chemicals as novel, potential selectively disruptive agents in tumor angiogenesis, consider the cross-hallmark relationships within tumor angiogenesis pathways and targets as well as with other cancer hallmarks and make suggestions for assessing environmental chemicals in tumor angiogenesis for future studies. Understanding of the role of low-dose exposure of chemicals with disruptive potential could help us to refine our approach to cancer risk assessment, and may ultimately aid in preventing cancer by reducing or eliminating exposures to synergistic mixtures of chemicals with carcinogenic potential.

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References

1. Hanahan, D. et al. (2000) The hallmarks of cancer. Cell, 100, 57–70.
2. Hanahan, D. et al. (2011) Hallmarks of cancer: the next generation. Cell, 144, 646–674.
3. Folkman, J. (1971) Tumor angiogenesis: therapeutic implications. N. Engl. J. Med., 285, 1182–1186.
4. Carmeliet, P. et al. (2011) Molecular mechanisms and clinical applications of angiogenesis. Nature, 473, 298–307.
5. Ferrara, N. et al. (2005) Angiogenesis as a therapeutic target. Nature, 438, 967–974.
6. Folkman, J. (2001) Angiogenesis-dependent diseases. Semin. Oncol., 28, 536–542.
7. Folkman, J. (2003) Angiogenesis inhibitors: a new class of drugs. Cancer Biol. Ther., 2(4 suppl. 1), S127–S133.
8. Cao, Y. et al. (2011) Forty-year journey of angiogenesis translational research. Sci. Transl. Med., 3, 114rv3.
9. Siemann, D.W. et al. (2005) Differentiation and definition of vascular-targeted therapies. Clin. Cancer Res., 11, 416–420.
10. Thorpe, P.E. (2004) Vascular targeting agents as cancer therapeutics. Clin. Cancer Res., 10, 415–427.
11. Patterson, D.M. et al. (2007) Vascular damaging agents. Clin. Oncol. (R. Coll. Radiol)., 19, 443–456.
12. Hu, Z. et al. (1999) Targeting tumor vasculature endothelial cells and tumor cells for immunotherapy of human melanoma in a mouse xenograft model. Proc. Natl. Acad. Sci. USA, 96, 8161–8166.
13. Hu, Z. et al. (2001) Targeting tissue factor on tumor vascular endothelial cells and tumor cells for immunotherapy in mouse models of prostatic cancer. Proc. Natl. Acad. Sci. USA, 98, 12180–12185.
14. Hu, Z. et al. (2010) Natural killer cells are crucial for the efficacy of Icon (factor VII/human IgG1 Fc) immunotherapy in human tongue cancer. BMC Immunol., 11, 49.
15. van Heeckeren, W.J. et al. (2007) Complications from vascular disrupting agents and angiogenesis inhibitors: aberrant control of hemostasis and thrombosis. Curr. Opin. Hematol., 14, 468–480.
16. Bair, S.M. et al. (2013) Cardiovascular complications associated with novel angiogenesis inhibitors: emerging evidence and evolving perspectives. Trends Cardiovasc. Med., 23, 104–113.
17. Ellis, L.M. et al. (2008) VEGF-targeted therapy: mechanisms of anti-tumour activity. Nat. Rev. Cancer, 8, 579–591.
18. Muncke, J. (2011) Release of chemicals from plastics: lessons from food contact with plastics. Integr. Environ. Assess. Manag., 7, 688–690.
19. Hecht, S.S. (2012) Lung carcinogenesis by tobacco smoke. Int. J. Cancer, 131, 2724–2732.
20. Letašiová, S. et al. (2012) Bladder cancer, a review of the environmental risk factors. Environ. Health, 11 (suppl. 1), S11.
21. Loeb, L.A. et al. (1984) Smoking and lung cancer: an overview. Cancer Res., 44(12 Pt 1), 5940–5958.
22. Pogribny, I.P. et al. (2013) Environmental toxicants, epigenetics, and cancer. Adv. Exp. Med. Biol., 754, 215–232.
23. Sankpal, U.T. et al. (2012) Environmental factors in causing human cancers: emphasis on tumorigenesis. Tumour Biol., 33, 1265–1274.
24. Torti, S.V. et al. (2013) Iron and cancer: more ore to be mined. Nat. Rev. Cancer, 13, 342–355.
25. Vandenberg, L.N. (2014) Low-dose effects of hormones and endocrine disruptors. Vitam. Horm., 94, 129–165.
26. Collotta, M. et al. (2013) Epigenetics and pesticides. Toxicology, 307, 35–41.
27. Cao, Y. (2005) Opinion: emerging mechanisms of tumour lymphangiogenesis and lymphatic metastasis. Nat. Rev. Cancer, 5, 735–743.
28. Risau, W. (1997) Mechanisms of angiogenesis. Nature, 386, 671–674.
29. Chung, A.S. et al. (2010) Targeting the tumour vasculature: insights from physiological angiogenesis. Nat. Rev. Cancer, 10, 505–514.

30. Potente, M. et al. (2011) Basic and therapeutic aspects of angiogenesis. Cell, 146, 873–887.
31. Weis, S.M. et al. (2011) Tumor angiogenesis: molecular pathways and therapeutic targets. Nat. Med., 17, 1359–1370.
32. Jain, R.K. et al. (2012) SnapShot: Tumor angiogenesis. Cell, 149, 1408–1408.e1.
33. Welti, J. et al. (2013) Recent molecular discoveries in angiogenesis and antiangiogenic therapies in cancer. J. Clin. Invest., 123, 3190–3200.
34. Kaelin, W.G. Jr. (2008) The von Hippel-Lindau tumour suppressor protein: O2 sensing and cancer. Nat. Rev. Cancer, 8, 865–873.
35. Semenza, G.L. (2009) Regulation of cancer cell metabolism by hypoxia-inducible factor 1. Semin. Cancer Biol., 19, 12–16.
36. Harris, A.L. (2002) Hypoxia–a key regulatory factor in tumour growth. Nat. Rev. Cancer, 2, 38–47.
37. Lewis, C.E. et al. (2007) Tie2-expressing monocytes and tumor angiogenesis: regulation by hypoxia and angiopoietin-2. Cancer Res., 67, 8429–8432.
38. Dvorak, H.F. (1986) Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N. Engl. J. Med., 315, 1650–1659.
39. Xue, Y. et al. (2012) PDGF-BB modulates hematopoiesis and tumor angiogenesis by inducing erythropoietin production in stromal cells. Nat. Med., 18, 100–110.
40. Avraamides, C.J. et al. (2008) Integrins in angiogenesis and lymphangiogenesis. Nat. Rev. Cancer, 8, 604–617.
41. Weis, S.M. et al. (2011) Tumor angiogenesis: molecular pathways and therapeutic targets. Nat. Med., 17, 1359–1370.
42. Singh, S. et al. (2007) Chemokines in tumor angiogenesis and metastasis. Cancer Metastasis Rev., 26, 453–467.
43. van Hinsbergh, V.W. et al. (2006) Pericellular proteases in angiogenesis and vasculogenesis. Arterioscler. Thromb. Vasc. Biol., 26, 716–728.
44. Wang, S.S. et al. (2009) AngiomiRs-Key regulators of angiogenesis. Curr. Opin. Genet. Dev., 19, 205–211.
45. Bao, L. et al. (2013) MicroRNA-21 suppresses PTEN and hSulf-1 expression and promotes hepatocellular carcinoma progression through AKT/ERK pathways. 2013 337(2):226–36.
46. Nagy, J.A. et al. (2007) VEGF-A and the induction of pathological angiogenesis. Annu. Rev. Pathol., 2, 251–275.
47. Jain, R.K. (2005) Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science, 307, 58–62.
48. Smith, N.R. et al. (2010) Vascular endothelial growth factor receptors VEGFR-2 and VEGFR-3 are localized primarily to the vasculature in human primary solid cancers. Clin. Cancer Res., 16, 3548–3561.
49. Christofori, G. et al. (1995) Vascular endothelial growth factor and its receptors, flt-1 and flk-1, are expressed in normal pancreatic islets and throughout islet cell tumorigenesis. Mol. Endocrinol., 9, 1760–1770.
50. Witmer, A.N. et al. (2002) Expression of vascular endothelial growth factor receptors 1, 2, and 3 in quiescent endothelia. J. Histochem. Cytochem., 50, 767–777.
51. Semeraro, N. et al. (1997) Tissue factor in health and disease. Thromb. Haemost., 78, 759–764.
52. Folkman, J. (1996) Tumor angiogenesis and tissue factor. Nat. Med., 2, 167–168.
53. Rickles, F.R. et al. (2001) The role of the hemostatic system in tumor growth, metastasis, and angiogenesis: tissue factor is a bifunctional molecule capable of inducing both fibrin deposition and angiogenesis in cancer. Int. J. Hematol., 73, 145–150.
54. Fernandez, P.M. et al. (2002) Tissue factor and angiogenesis in cancer. Curr. Opin. Hematol., 9, 401–406.
55. Pawlinski, R. et al. (2004) Role of tissue factor in haemostasis, thrombosis, angiogenesis and inflammation: lessons from low tissue factor mice. Thromb. Haemost., 92, 444–450.
56. Rak, J. et al. (2006) Tissue factor in cancer and angiogenesis: the molecular link between genetic tumor progression, tumor neovascularization, and cancer coagulopathy. Semin. Thromb. Hemost., 32, 54–70.
57. Fisher, K.L. et al. (1987) Cloning and expression of human tissue factor cDNA. Thromb. Res., 48, 89–99.
58. Morrissey, J.H. et al. (1987) Molecular cloning of the cDNA for tissue factor, the cellular receptor for the initiation of the coagulation protease cascade. Cell, 50, 129–135.
59. Spicer, E.K. et al. (1987) Isolation of cDNA clones coding for human tissue factor: primary structure of the protein and cDNA. Proc. Natl. Acad. Sci. USA, 84, 5148–5152.
60. Konigsberg, W.H. et al. (1988) Molecular cloning of the cDNA for human tissue factor. Cell, 52, 639–640.
61. O'Hara, P.J. et al. (1987) Nucleotide sequence of the gene coding for human factor VII, a vitamin K-dependent protein participating in blood coagulation. Proc. Natl. Acad. Sci. USA, 84, 5158–5162.
62. Idusogie, E. et al. (1996) Characterization of a cDNA encoding murine coagulation factor VII. Thromb. Haemost., 75, 481–487.
63. Nemerson, Y. (1983) Regulation of the initiation of coagulation by factor VII. Haemostasis, 13, 150–155.
64. Contrino, J. et al. (1996) In situ detection of tissue factor in vascular endothelial cells: correlation with the malignant phenotype of human breast disease. Nat. Med., 2, 209–215.
65. Duanmu, J. et al. (2011) Effective treatment of chemoresistant breast cancer *in vitro* and *in vivo* by a factor VII-targeted photodynamic therapy. Br. J. Cancer, 104, 1401–1409.
66. Shoji, M. et al. (1998) Activation of coagulation and angiogenesis in cancer: immunohistochemical localization in situ of clotting proteins and vascular endothelial growth factor in human cancer. Am. J. Pathol., 152, 399–411.
67. Gabrilovich, D.I. et al. (1996) Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. Nat. Med., 2, 1096–1103.
68. Goel, H.L. et al. (2013) VEGF targets the tumour cell. Nat. Rev. Cancer, 13, 871–882.
69. Yao, X.H. et al. (2008) Glioblastoma stem cells produce vascular endothelial growth factor by activation of a G-protein coupled formylpeptide receptor FPR. J. Pathol., 215, 369–376.
70. Abdulkadir, S.A. et al. (2000) Tissue factor expression and angiogenesis in human prostate carcinoma. Hum. Pathol., 31, 443–447.
71. Zucker, S. et al. (1998) Vascular endothelial growth factor induces tissue factor and matrix metalloproteinase production in endothelial cells: conversion of prothrombin to thrombin results in progelatinase A activation and cell proliferation. Int. J. Cancer, 75, 780–786.
72. Hu, Z. et al. (2010) Targeting tissue factor on tumour cells and angiogenic vascular endothelial cells by factor VII-targeted verteporfin photodynamic therapy for breast cancer *in vitro* and *in vivo* in mice. BMC Cancer, 10, 235.
73. Hu, Z. (2011) Factor VII-targeted photodynamic therapy for breast cancer and its therapeutic potential for other solid cancers and leukemia. Breast Cancer - Current and Alternative Therapeutic Modalities, Prof. Esra Gunduz (Ed.), pp.175–196. ISBN: 978-953-307-776-5, InTech, doi:10.5772/20398.
74. Ruf, W. et al. (2010) Tissue factor in cancer progression and angiogenesis. Thromb. Res., 125 (suppl. 2), S36–S38.
75. Belting, M. et al. (2004) Regulation of angiogenesis by tissue factor cytoplasmic domain signaling. Nat. Med., 10, 502–509.
76. Fernandez, P.M. et al. (2004) Tissue factor and fibrin in tumor angiogenesis. Semin. Thromb. Hemost., 30, 31–44.
77. Versteeg, H.H. et al. (2003) Tissue factor signal transduction in angiogenesis. Carcinogenesis, 24, 1009–1013.
78. Kleinstreuer, N.C. et al. (2013) *In vitro* perturbations of targets in cancer hallmark processes predict rodent chemical carcinogenesis. Toxicol. Sci., 131, 40–55.
79. Vicari, A.P. et al. (2002) Chemokines in cancer. Cytokine Growth Factor Rev., 13, 143–154.
80. Romagnani, P. et al. (2004) CXC chemokines: the regulatory link between inflammation and angiogenesis. Trends Immunol., 25, 201–209.
81. Horowitz, N.A. et al. (2011) Thrombomodulin is a determinant of metastasis through a mechanism linked to the thrombin binding domain but not the lectin-like domain. Blood, 118, 2889–2895.
82. Liu, P.L. et al. (2010) Decreased expression of thrombomodulin is correlated with tumor cell invasiveness and poor prognosis in nonsmall cell lung cancer. Mol. Carcinog., 49, 874–881.
83. Tang, X. et al. (2012) p53 is an important regulator of CCL2 gene expression. Curr. Mol. Med., 12, 929–943.
84. Lin, T.H. et al. (2013) CCL2 increases alphavbeta3 integrin expression and subsequently promotes prostate cancer migration. Biochim. Biophys. Acta, 1830, 4917–4927.

85. Gorgoulis, V.G. et al. (2003) p53 activates ICAM-1 (CD54) expression in an NF-kappaB-independent manner. EMBO J., 22, 1567–1578.
86. Zhang, H.M. et al. (2005) Gamma interferon-inducible protein 10 induces HeLa cell apoptosis through a p53-dependent pathway initiated by suppression of human papillomavirus type 18 E6 and E7 expression. Mol. Cell. Biol., 25, 6247–6258.
87. O'Donnell, E.F. et al. (2014) The aryl hydrocarbon receptor mediates raloxifene-induced apoptosis in estrogen receptor-negative hepatoma and breast cancer cells. Cell Death Dis., 5, e1038.
88. Kung, T. et al. (2009) The aryl hydrocarbon receptor (AhR) pathway as a regulatory pathway for cell adhesion and matrix metabolism. Biochem. Pharmacol., 177, 11.
89. Dietrich, C. et al. (2010) The aryl hydrocarbon receptor (AhR) in the regulation of cell-cell contact and tumor growth. Carcinogenesis, 31, 1319–1328.
90. Andrysík, Z. et al. (2007) The aryl hydrocarbon receptor-dependent deregulation of cell cycle control induced by polycyclic aromatic hydrocarbons in rat liver epithelial cells. Mutat. Res., 615, 87–97.
91. Weiss, C. et al. (2008) TCDD deregulates contact inhibition in rat liver oval cells via Ah receptor, JunD and cyclin A. Oncogene, 27, 2198–2207.
92. Roman, A.C. et al. (2009) Dioxin receptor deficiency impairs angiogenesis by a mechanism involving VEGF-A depletion in the endothelium and transforming growth factor-beta overexpression in the stroma. J. Biol. Chem., 284, 25135–25148.
93. Feng, S. et al. (2013) Role of aryl hydrocarbon receptor in cancer. Biochim Biophys Acta, 1836, 14.
94. Huang, H.C. et al. (2013) UVB irradiation regulates ERK1/2- and p53-dependent thrombomodulin expression in human keratinocytes. PLoS One, 8, e67632.
95. Kumar, A. et al. (2011) p53 impairs endothelial function by transcriptionally repressing Kruppel-like factor 2. Arterioscler. Thromb. Vasc. Biol., 31, 133–141.
96. Maekita, T. et al. (2006) High levels of aberrant DNA methylation in Helicobacter pylori-infected gastric mucosae and its possible association with gastric cancer risk. Clin. Cancer Res., 12(3 Pt 1), 989–995.
97. Lengauer, C. et al. (1997) DNA methylation and genetic instability in colorectal cancer cells. Proc. Natl. Acad. Sci. USA, 94, 2545–2550.
98. Ikezoe, T. et al. (2012) Thrombomodulin protects endothelial cells from a calcineurin inhibitor-induced cytotoxicity by upregulation of extracellular signal-regulated kinase/myeloid leukemia cell-1 signaling. Arterioscler. Thromb. Vasc. Biol., 32, 2259–2270.
99. Pickard, A. et al. (2012) Inactivation of Rb in stromal fibroblasts promotes epithelial cell invasion. EMBO J., 31, 3092–3103.
100. Kim, S. et al. (2008) Basal and UV-induced MMP-1 expression are inhibited by p53 in human dermal fibroblasts. Exp. Dermatol., 17, 939–945.
101. Wu, Y.H. et al. (2010) p53 dysfunction by xeroderma pigmentosum group C defects enhance lung adenocarcinoma metastasis via increased MMP1 expression. Cancer Res., 70, 10422–10432.
102. Singh, P.K. et al. (2008) Phosphorylation of MUC1 by Met modulates interaction with p53 and MMP1 expression. J. Biol. Chem., 283, 26985–26995.
103. Gopinath, S. et al. (2010) Co-depletion of cathepsin B and uPAR induces G0/G1 arrest in glioma via FOXO3a mediated p27 upregulation. PLoS One, 5, e11668.
104. Doll, R. (1986) Tobacco: an overview of health effects. IARC Scientific Publications, pp. 11–22.
105. Costa, F. et al. (2009) Nicotine: a pro-angiogenic factor. Life Sci., 84, 785–790.
106. Hecht, S.S. et al. (1975) Chemical studies on tobacco smoke. XXXIII. N' -nitrosonornicotine in tobacco: analysis of possible contributing factors and biologic implications. J. Natl. Cancer Inst., 54, 1237–1244.
107. Liu, E.S. et al. (2003) Cigarette smoke exposure increases ulcerative colitis-associated colonic adenoma formation in mice. Carcinogenesis, 24, 1407–1413.
108. Zhu, B.Q. et al. (2003) Second hand smoke stimulates tumor angiogenesis and growth. Cancer Cell, 4, 191–196.
109. Wang, Y.H. et al. (2013) Comparing the joint effect of arsenic exposure, cigarette smoking and risk genotypes of vascular endothelial growth factor on upper urinary tract urothelial carcinoma and bladder cancer. J. Hazard. Mater 262:1139–46.
110. Shin, V.Y. et al. (2004) Nicotine promotes gastric tumor growth and neovascularization by activating extracellular signal-regulated kinase and cyclooxygenase-2. Carcinogenesis, 25, 2487–2495.
111. Zhang, Q. et al. (2007) Nicotine induces hypoxia-inducible factor-1alpha expression in human lung cancer cells via nicotinic acetylcholine receptor-mediated signaling pathways. Clin. Cancer Res., 13, 4686–4694.
112. Park, Y.J. et al. (2008) Effect of nicotine on human umbilical vein endothelial cells (HUVECs) migration and angiogenesis. Vascul. Pharmacol., 49, 32–36.
113. Heeschen, C. et al. (2001) Nicotine stimulates angiogenesis and promotes tumor growth and atherosclerosis. Nat. Med., 7, 833–839.
114. Wong, H.P. et al. (2007) Nicotine promotes colon tumor growth and angiogenesis through beta-adrenergic activation. Toxicol. Sci., 97, 279–287.
115. Mousa, S. et al. (2006) Cellular and molecular mechanisms of nicotine's pro-angiogenesis activity and its potential impact on cancer. J. Cell. Biochem., 97, 1370–1378.
116. Martin, J.W. et al. (2009) The multiple faces of nicotine and its implications in tissue and wound repair. Exp. Dermatol., 18, 497–505.
117. Kao, Y.H. et al. (2003) Low concentrations of arsenic induce vascular endothelial growth factor and nitric oxide release and stimulate angiogenesis *in vitro*. Chem. Res. Toxicol., 16, 460–468.
118. Klei, L.R. et al. (2008) Positive signaling interactions between arsenic and ethanol for angiogenic gene induction in human microvascular endothelial cells. Toxicol. Sci., 102, 319–327.
119. Straub, A.C. et al. (2009) Arsenic requires sphingosine-1-phosphate type 1 receptors to induce angiogenic genes and endothelial cell remodeling. Am. J. Pathol., 174, 1949–1958.
120. Meng, D. et al. (2010) Arsenic promotes angiogenesis *in vitro* via a heme oxygenase-1-dependent mechanism. Toxicol. Appl. Pharmacol., 244, 291–299.
121. Straub, A.C. et al. (2007) Low level arsenic promotes progressive inflammatory angiogenesis and liver blood vessel remodeling in mice. Toxicol. Appl. Pharmacol., 222, 327–336.
122. Xu, X. et al. (2009) Diesel exhaust exposure induces angiogenesis. Toxicol. Lett., 191, 57–68.
123. Andersson, H. et al. (2012) Proangiogenic effects of environmentally relevant levels of bisphenol A in human primary endothelial cells. Arch. Toxicol., 86, 465–474.
124. Bredow, S. et al. (2007) Subchronic inhalation of soluble manganese induces expression of hypoxia-associated angiogenic genes in adult mouse lungs. Toxicol. Appl. Pharmacol., 221, 148–157.
125. Ishimura, R. et al. (2009) Dioxin-induced toxicity on vascular remodeling of the placenta. Biochem. Pharmacol., 77, 660–669.
126. Halin, C. et al. (2008) Chapter 1. Inflammation, angiogenesis, and lymphangiogenesis. Methods Enzymol., 445, 1–25.
127. Tice, R.R. et al. (2013) Improving the human hazard characterization of chemicals: a Tox21 update. Environ. Health Perspect., 121, 756–765.
128. Kavlock, R. et al. (2012) Update on EPA's ToxCast program: providing high throughput decision support tools for chemical risk management. Chem. Res. Toxicol., 25, 1287–1302.
129. Martin, M.T. et al. (2009) Profiling the reproductive toxicity of chemicals from multigeneration studies in the toxicity reference database. Toxicol. Sci., 110, 181–190.
130. Judson, R.S. et al. (2010) *In vitro* screening of environmental chemicals for targeted testing prioritization: the ToxCast project. Environ. Health Perspect., 118, 485–492.
131. Dairkee, S.H. et al. (2013) Bisphenol-A-induced inactivation of the p53 axis underlying deregulation of proliferation kinetics, and cell death in non-malignant human breast epithelial cells. Carcinogenesis, 34, 703–712.
132. Goldman, J.M. et al. (2004) Methoxychlor-induced alterations in the histological expression of angiogenic factors in pituitary and uterus. J. Mol. Histol., 35, 363–375.

133. Qian, Y. et al. (2010) Perfluorooctane sulfonate (PFOS) induces reactive oxygen species (ROS) production in human microvascular endothelial cells: role in endothelial permeability. J. Toxicol. Environ. Health. A, 73, 819–836.
134. Zhang, Y.H. et al. (2013) Mechanism of perfluorooctanesulfonate (PFOS)-induced apoptosis in the immunocyte. J. Immunotoxicol., 10, 49–58.
135. Jessen-Eller, K. et al. (2002) A new invertebrate member of the p53 gene family is developmentally expressed and responds to polychlorinated biphenyls. Environ. Health Perspect., 110, 377–385.
136. Taylor, T.R. et al. (2011) Ziram activates mitogen-activated protein kinases and decreases cytolytic protein levels in human natural killer cells. Toxicol. Mech. Methods, 21, 577–584.
137. Wu, P.Y. et al. (2013) Aromatic hydrocarbon receptor inhibits lysophosphatidic acid-induced vascular endothelial growth factor-A expression in PC-3 prostate cancer cells. Biochem. Biophys. Res. Commun., 437, 440–445.
138. Madri, J.A. et al. (1986) Endothelial cell-matrix interactions: *in vitro* models of angiogenesis. J. Histochem. Cytochem., 34, 85–91.
139. Carmeliet, P. et al. (1998) Mouse models of angiogenesis, arterial stenosis, atherosclerosis and hemostasis. Cardiovasc. Res., 39, 8–33.
140. Vailhé, B. et al. (2001) *In vitro* models of vasculogenesis and angiogenesis. Lab. Invest., 81, 439–452.
141. Bernardini, G. et al. (2004) *In vitro* and *in vivo* models to study chemokine regulation of angiogenesis. Methods Mol. Biol., 239, 223–232.
142. Radovanovic, I. et al. (2004) Angiogenesis in transgenic models of multistep angiogenesis. Cancer Treat. Res., 117, 97–114.
143. Hoang, M.V. et al. (2005) *In vivo* and *in vitro* models of Mammalian angiogenesis. Methods Mol. Biol., 294, 269–285.
144. Norrby, K. (2006) *In vivo* models of angiogenesis. J. Cell. Mol. Med., 10, 588–612.
145. Ucuzian, A.A. et al. (2007) *In vitro* models of angiogenesis. World J. Surg., 31, 654–663.
146. Deryugina, E.I. et al. (2008) Chapter 2. Chick embryo chorioallantoic membrane models to quantify angiogenesis induced by inflammatory and tumor cells or purified effector molecules. Methods Enzymol., 444, 21–41.
147. Shiba, Y. et al. (2008) Models for the study of angiogenesis. Curr. Pharm. Des., 14, 371–377.
148. Jensen, L.D. et al. (2009) *In vivo* angiogenesis and lymphangiogenesis models. Curr. Mol. Med., 9, 982–991.
149. Qutub, A.A. et al. (2009) Multiscale models of angiogenesis. IEEE Eng. Med. Biol. Mag., 28, 14–31.
150. Eklund, L. et al. (2013) Mouse models for studying angiogenesis and lymphangiogenesis in cancer. Mol. Oncol., 7, 259–282.
151. Morin, K.T. et al. (2013) In vitro models of angiogenesis and vasculogenesis in fibrin gel. Exp Cell Res 319(16):2409–17.
152. Swartz, M.A. et al. (2012) Tumor microenvironment complexity: emerging roles in cancer therapy. Cancer Res., 72, 2473–2480.
153. Sounni, N.E. et al. Targeting the tumor microenvironment for cancer therapy. Clin. Chem., 59, 85–93.
154. Shang, B. et al. (2012) Deciphering the key features of malignant tumor microenvironment for anti-cancer therapy. Cancer Microenviron., 5, 211–223.
155. Kwee, B.J. et al. (2014) Manipulating the intersection of angiogenesis and inflammation. Ann. Biomed. Eng.
156. Schweighofer, B. et al. (2009) The VEGF-induced transcriptional response comprises gene clusters at the crossroad of angiogenesis and inflammation. Thromb. Haemost., 102, 544–554.
157. Kobayashi, H. et al. (2009) Angiogenesis links chronic inflammation with cancer. Methods Mol. Biol., 511, 185–191.
158. Alfonso, L. et al. (2014) Molecular targets of aspirin and cancer prevention. Br. J. Cancer, 111, 61–67.
159. Gronich, N. et al. (2013) Beyond aspirin-cancer prevention with statins, metformin and bisphosphonates. Nat. Rev. Clin. Oncol., 10, 625–642.
160. Borthwick, G.M. et al. (2006) Therapeutic levels of aspirin and salicylate directly inhibit a model of angiogenesis through a Cox-independent mechanism. FASEB J., 20, 2009–2016.
161. Etulain, J. et al. (2013) Platelet-mediated angiogenesis is independent of VEGF and fully inhibited by aspirin. Br. J. Pharmacol., 170, 255–265.
162. Singh, A.V. et al. (2006) Soy phytochemicals prevent orthotopic growth and metastasis of bladder cancer in mice by alterations of cancer cell proliferation and apoptosis and tumor angiogenesis. Cancer Res., 66, 1851–1858.
163. Kim, E.Y. et al. (2008) A novel integrin alpha5beta1 antagonistic peptide, A5-1, screened by Protein Chip system as a potent angiogenesis inhibitor. Biochem. Biophys. Res. Commun., 377, 1288–1293.
164. Peterson, K.J. et al. (2008) A fluorescence polarization assay for identifying ligands that bind to vascular endothelial growth factor. Anal. Biochem., 378, 8–14.
165. Wang, K. et al. (2011) Development of an AlphaScreen-based high-throughput screening assay for inhibitors of human vascular endothelial growth factor receptor-3. J. Immunoassay Immunochem., 32(3):219–232.
166. Moshinsky, D.J. et al. (2003) A widely applicable, high-throughput TR-FRET assay for the measurement of kinase autophosphorylation: VEGFR-2 as a prototype. J. Biomol. Screen., 8, 447–452.
167. Santos, A.F. et al. (2008) Angiogenesis: an improved *in vitro* biological system and automated image-based workflow to aid identification and characterization of angiogenesis and angiogenic modulators. Assay Drug Dev. Technol., 6, 693–710.
168. Wang, H.S. et al. (2004) A simple quantitative method for evaluation of angiogenesis activity. Assay Drug Dev. Technol., 2(1):31–38.
169. Reed, M.J. et al. (2007) Culture of murine aortic explants in 3-dimensional extracellular matrix: a novel, miniaturized assay of angiogenesis *in vitro*. Microvasc. Res., 73, 248–252.
170. Sanz, L. et al. (2002) Development of a computer-assisted high-throughput screening platform for anti-angiogenic testing. Microvasc. Res., 63, 335–339.
171. Yauch, R.L. et al. (2004) Transcriptional-based screens for pathway-specific, high-throughput target discovery in endothelial cells. J. Biomol. Screen., 9, 704–711.
172. Bischel, L.L. et al. (2013) Tubeless microfluidic angiogenesis assay with three-dimensional endothelial-lined microvessels. Biomaterials, 34(5):1471–1477.
173. Yarrow, J.C. et al. (2004) A high-throughput cell migration assay using scratch wound healing, a comparison of image-based readout methods. BMC Biotechnol., 4, 21.
174. Kumar, N. et al. (2006) A high-throughput migration assay reveals HER2-mediated cell migration arising from increased directional persistence. Biophys. J., 91, L32–L34.
175. Huynh, Q.K. et al. (2011) Screening and identification of a novel class of TGF-beta type 1 receptor kinase inhibitor. J. Biomol. Screen., 16, 724–33.
176. Kung, A.L. et al. (2004) Small molecule blockade of transcriptional coactivation of the hypoxia-inducible factor pathway. Cancer Cell, 6, 33–43.
177. Lee, Y. et al. (2004) High-throughput screening of novel peptide inhibitors of an integrin receptor from the hexapeptide library by using a protein microarray chip. J. Biomol. Screen., 9, 687–694.
178. Staton, C.A. et al. (2009) A critical analysis of current *in vitro* and *in vivo* angiogenesis assays. Int. J. Exp. Pathol., 90, 195–221.
179. Evensen, L. et al. (2010) A novel imaging-based high-throughput screening approach to anti-angiogenic drug discovery. Cytometry. A, 77, 41–51.
180. Ji, D.B. et al. (2008) Establishment of a cell-based assay to screen regulators of the hypoxia-inducible factor-1-dependent vascular endothelial growth factor promoter. Biol. Pharm. Bull., 31, 2255–2259.
181. Johnson, R.L. et al. (2009) A quantitative high-throughput screen for modulators of IL-6 signaling: a model for interrogating biological networks using chemical libraries. Mol. Biosyst., 5, 1039–1050.
182. Xia, M. et al. (2009) Identification of chemical compounds that induce HIF-1alpha activity. Toxicol. Sci., 112, 153–163.
183. Smirnova, N.A. et al. (2010) Utilization of an *in vivo* reporter for high throughput identification of branched small molecule regulators of hypoxic adaptation. Chem. Biol., 17, 380–391.
184. Miller, S.C. et al. (2010) Identification of known drugs that act as inhibitors of NF-kappaB signaling and their mechanism of action. Biochem. Pharmacol., 79, 1272–1280.

185. Takahashi, T. et al. (2011) An *in vitro* test to screen skin sensitizers using a stable THP-1-derived IL-8 reporter cell line, THP-G8. Toxicol. Sci., 124, 359–369.
186. Cash, J.N. et al. (2013) Development of a small-molecule screening method for inhibitors of cellular response to myostatin and activin A. J. Biomol. Screen.
187. Li, X. et al. (2009) Identification of upregulators of BMP2 expression via high-throughput screening of a synthetic and natural compound library. J. Biomol. Screen., 14, 1251–1256.
188. Shukla, S.J. et al. (2010) The future of toxicity testing: a focus on *in vitro* methods using a quantitative high-throughput screening platform. Drug Discov. Today, 15, 997–1007.
189. Attene-Ramos, M.S. et al. (2013) The Tox21 robotic platform for the assessment of environmental chemicals - from vision to reality. Drug Discov. Today.
190. Dejardin, E. (2006) The alternative NF-kappaB pathway from biochemistry to biology: pitfalls and promises for future drug development. Biochem. Pharmacol., 72, 1161–1179.
191. Chen, L.M. et al. (2011) RANKL increases migration of human lung cancer cells through intercellular adhesion molecule-1 up-regulation. J. Cell. Biochem., 112, 933–941.
192. Liu, S.Q. et al. (2013) Sphingosine kinase 1 promotes tumor progression and confers malignancy phenotypes of colon cancer by regulating the focal adhesion kinase pathway and adhesion molecules. Int. J. Oncol., 42, 617–626.
193. Madhavan, M. et al. (2002) Down regulation of endothelial adhesion molecules in node positive breast cancer: possible failure of host defence mechanism. Pathol. Oncol. Res., 8, 125–128.
194. Ding, Y.B. et al. (2003) Association of VCAM-1 overexpression with oncogenesis, tumor angiogenesis and metastasis of gastric carcinoma. World J. Gastroenterol., 9, 1409–1414.
195. Heidemann, J. et al. (2006) Expression of vascular cell adhesion molecule-1 (CD 106) in normal and neoplastic human esophageal squamous epithelium. Int. J. Oncol., 28, 77–85.
196. Slack-Davis, J.K. et al. (2009) Vascular cell adhesion molecule-1 is a regulator of ovarian cancer peritoneal metastasis. Cancer Res., 69, 1469–1476.
197. Yepuri, G. et al. (2012) Positive crosstalk between arginase-II and S6K1 in vascular endothelial inflammation and aging. Aging Cell, 11, 1005–1016.
198. Bose, A. et al. (2013) Tumor-derived vascular pericytes anergize Th cells. J. Immunol., 191, 971–981.
199. Petro, M. et al. (2013) Cutaneous tumors cease CXCL9/Mig production as a result of IFN-γ-mediated immunoediting. J. Immunol., 190, 832–841.
200. Kryczek, I. et al. (2009) Phenotype, distribution, generation, and functional and clinical relevance of Th17 cells in the human tumor environments. Blood, 114, 1141–1149.
201. Chang, K.P. et al. (2013) Serum levels of chemokine (C-X-C motif) ligand 9 (CXCL9) are associated with tumor progression and treatment outcome in patients with oral cavity squamous cell carcinoma. Oral Oncol., 49, 802–807.
202. Walser, T.C. et al. (2007) Immune-mediated modulation of breast cancer growth and metastasis by the chemokine Mig (CXCL9) in a murine model. J. Immunother., 30, 490–498.
203. Reckamp, K.L. et al. (2007) Expression of CXCR3 on mononuclear cells and CXCR3 ligands in patients with metastatic renal cell carcinoma in response to systemic IL-2 therapy. J. Immunother., 30, 417–424.
204. Bronger, H. et al. (2012) Modulation of CXCR3 ligand secretion by prostaglandin E2 and cyclooxygenase inhibitors in human breast cancer. Breast Cancer Res., 14, R30.
205. O'Garra, A. et al. (2012) Editorial overview. Curr. Opin. Immunol., 24, 361–363.
206. Musah, S. et al. (2012) Tumor necrosis factor-α mediates interactions between macrophages and epithelial cells underlying proinflammatory gene expression induced by particulate matter. Toxicology, 299, 125–132.
207. Wong, J.L. et al. (2013) IL-18-primed helper NK cells collaborate with dendritic cells to promote recruitment of effector CD8+ T cells to the tumor microenvironment. Cancer Res., 73, 4653–4662.
208. Hanly, A.M. et al. (2006) Thrombomodulin expression in colorectal carcinoma is protective and correlates with survival. Br. J. Cancer, 94, 1320–1325.
209. Chen, L.M. et al. (2013) Thrombomodulin mediates the progression of epithelial ovarian cancer cells. Tumour Biol., 34, 3743–3751.
210. Tai, C.J. et al. (2014) Thrombomodulin mediates the migration of cervical cancer cells through the regulation of epithelial-mesenchymal transition biomarkers. Tumour Biol., 35, 47–54.
211. Rajashekhar, G. et al. (2012) Soluble thrombomodulin reduces inflammation and prevents microalbuminuria induced by chronic endothelial activation in transgenic mice. Am. J. Physiol. Renal Physiol., 302, F703–F712.
212. Joven, J. et al. (2013) Multifunctional targets of dietary polyphenols in disease: a case for the chemokine network and energy metabolism. Food Chem. Toxicol., 51, 267–279.
213. Hacke, K. et al. (2010) Regulation of MCP-1 chemokine transcription by p53. Mol. Cancer, 9, 82.
214. Redon, C.E. et al. (2010) Tumors induce complex DNA damage in distant proliferative tissues *in vivo*. Proc. Natl. Acad. Sci. USA, 107, 17992–17997.
215. Kudo-Saito, C. et al. (2013) CCL2 is critical for immunosuppression to promote cancer metastasis. Clin. Exp. Metastasis, 30, 393–405.
216. Huang, B. et al. (2007) CCL2/CCR2 pathway mediates recruitment of myeloid suppressor cells to cancers. Cancer Lett., 252, 86–92.
217. Eugenin, E.A. et al. (2003) MCP-1 (CCL2) protects human neurons and astrocytes from NMDA or HIV-tat-induced apoptosis. J. Neurochem., 85, 1299–1311.
218. Hu, W.T. et al. (2014) IL-33 enhances proliferation and invasiveness of decidual stromal cells by up-regulation of CCL2/CCR2 via NF-κB and ERK1/2 signaling. Mol. Hum. Reprod., 20, 358–372.
219. Soria, G. et al. (2011) Inflammatory mediators in breast cancer: coordinated expression of TNFalpha & IL-1beta with CCL2 & CCL5 and effects on epithelial-to-mesenchymal transition. BMC Cancer, 11, 130.
220. Li, M.Q. et al. (2011) CD82 gene suppression in endometrial stromal cells leads to increase of the cell invasiveness in the endometriotic milieu. J. Mol. Endocrinol., 47, 195–208.
221. Izumi, K. et al. (2013) Targeting the androgen receptor with siRNA promotes prostate cancer metastasis through enhanced macrophage recruitment via CCL2/CCR2-induced STAT3 activation. EMBO Mol. Med., 5, 1383–1401.
222. Karasawa, F. et al. (2012) Essential role of gastric gland mucin in preventing gastric cancer in mice. J. Clin. Invest., 122, 923–934.
223. Borsig, L. et al. (2014) Inflammatory chemokines and metastasis–tracing the accessory. Oncogene, 33, 3217–3224.
224. Fang, W. et al. (2005) The effects of polygoni multiflori total glycosides on the experimentally atherosclerotic formation in apoE-deficient mice. Zhongguo Zhong Yao Za Zhi, 30, 1542–1545.
225. Krutmann, J. et al. (1994) Evidence that DNA damage is a mediate in ultraviolet B radiation-induced inhibition of human gene expression: ultraviolet B radiation effects on intercellular adhesion molecule-1 (ICAM-1) expression. J. Invest. Dermatol., 102, 428–432.
226. Shirai, A. et al. (2003) Expression of intercellular adhesion molecule (ICAM)-1 in adenoid cystic carcinoma of the head and neck. Laryngoscope, 113, 1955–1960.
227. Gallaher, A.M. et al. (2013) Proteomic screening of human targets of viral microRNAs reveals functions associated with immune evasion and angiogenesis. PLoS Pathog., 9, e1003584.
228. Li, X. et al. (2010) The helix-loop-helix transcription factor TWIST is dysregulated in myelodysplastic syndromes. Blood, 116, 2304–2314.
229. Usami, Y. et al. (2013) Intercellular adhesion molecule-1 (ICAM-1) expression correlates with oral cancer progression and induces macrophage/cancer cell adhesion. Int. J. Cancer, 133, 568–578.
230. Schröder, C. et al. (2011) Prognostic value of intercellular adhesion molecule (ICAM)-1 expression in breast cancer. J. Cancer Res. Clin. Oncol., 137, 1193–1201.
231. Buitrago, D. et al. (2012) Intercellular adhesion molecule-1 (ICAM-1) is upregulated in aggressive papillary thyroid carcinoma. Ann. Surg. Oncol., 19, 973–980.

232. Ramer, R. et al. (2012) Cannabidiol inhibits lung cancer cell invasion and metastasis via intercellular adhesion molecule-1. FASEB J., 26, 1535–1548.
233. Rafat, M. et al. (2012) Engineered endothelial cell adhesion via VCAM1 and E-selectin antibody-presenting alginate hydrogels. Acta Biomater., 8, 2697–2703.
234. Oberyszyn, T.M. et al. (1998) Beta2 integrin/ICAM-1 adhesion molecule interactions in cutaneous inflammation and tumor promotion. Carcinogenesis, 19, 445–455.
235. Chen, Q. et al. (2012) Molecular pathways: VCAM-1 as a potential therapeutic target in metastasis. Clin. Cancer Res., 18, 5520–5525.
236. Grismayer, B. et al. (2012) Overexpression of the urokinase receptor splice variant uPAR-del4/5 in breast cancer cells affects cell adhesion and invasion in a dose-dependent manner and modulates transcription of tumor-associated genes. Biol. Chem., 393, 1449–1455.
237. Shetty, S. et al. (2007) Regulation of urokinase receptor expression by p53: novel role in stabilization of uPAR mRNA. Mol. Cell. Biol., 27, 5607–5618.
238. Shetty, P. et al. (2008) Urokinase expression by tumor suppressor protein p53: a novel role in mRNA turnover. Am. J. Respir. Cell Mol. Biol., 39, 364–372.
239. Gupta, R. et al. (2011) Oncogenic role of p53 is suppressed by si-RNA bicistronic construct of uPA, uPAR and cathepsin-B in meningiomas both *in vitro* and *in vivo*. Int. J. Oncol., 38, 973–983.
240. Wang, F. et al. (2011) Virtual screening targeting the urokinase receptor, biochemical and cell-based studies, synthesis, pharmacokinetic characterization, and effect on breast tumor metastasis. J. Med. Chem., 54, 7193–7205.
241. Hodjat, M. et al. (2013) Urokinase receptor mediates doxorubicin-induced vascular smooth muscle cell senescence via proteasomal degradation of TRF2. J. Vasc. Res., 50, 109–123.
242. Bhandary, Y.P. et al. (2013) Regulation of lung injury and fibrosis by p53-mediated changes in urokinase and plasminogen activator inhibitor-1. Am. J. Pathol., 183, 131–143.
243. Testa, J.E. et al. (1990) The role of urokinase-type plasminogen activator in aggressive tumor cell behavior. Cancer Metastasis Rev., 9, 353–367.
244. Stahl, A. et al. (1994) Binding of urokinase to its receptor promotes migration and invasion of human melanoma cells *in vitro*. Cancer Res., 54, 3066–3071.
245. Cantero, D. et al. (1997) Enhanced expression of urokinase plasminogen activator and its receptor in pancreatic carcinoma. Br. J. Cancer, 75, 388–395.
246. Reuning, U. et al. (1998) Multifunctional potential of the plasminogen activation system in tumor invasion and metastasis (review). Int. J. Oncol., 13, 893–906.
247. Lund, I.K. et al. (2011) uPAR as anti-cancer target: evaluation of biomarker potential, histological localization, and antibody-based therapy. Curr. Drug Targets, 12, 1744–1760.
248. Noh, H. et al. (2013) Role of urokinase receptor in tumor progression and development. Theranostics, 3, 487–495.
249. Thunø, M. et al. (2009) suPAR: the molecular crystal ball. Dis. Markers, 27, 157–172.
250. Zhuang, T. et al. (2013) Involvement of nitric oxide synthase in matrix metalloproteinase-9- and/or urokinase plasminogen activator receptor-mediated glioma cell migration. BMC Cancer, 13, 590.
251. Marcus, H.J. et al. (2010) *In vivo* assessment of high-grade glioma biochemistry using microdialysis: a study of energy-related molecules, growth factors and cytokines. J. Neurooncol., 97, 11–23.
252. Wapnir, I.L. et al. (1996) Collagen gene expression in the neomatrix of carcinoma of the breast. Invas. Metastasis, 16, 308–316.
253. Menke, A. et al. (2001) Down-regulation of E-cadherin gene expression by collagen type I and type III in pancreatic cancer cell lines. Cancer Res., 61, 3508–3517.
254. Imamichi, Y. et al. (2007) Signaling pathways involved in collagen-induced disruption of the E-cadherin complex during epithelial-mesenchymal transition. Cells. Tissues. Organs, 185, 180–190.
255. Zhang, X. et al. (2013) Histone deacetylase inhibition downregulates collagen 3A1 in fibrotic lung fibroblasts. Int. J. Mol. Sci., 14, 19605–19617.
256. Boyd, A. et al. (2013) Quiescent innate response to infective filariae by human Langerhans cells suggests a strategy of immune evasion. Infect. Immun., 81, 1420–1429.
257. Srivastava, M.K. et al. (2012) Myeloid suppressor cell depletion augments antitumor activity in lung cancer. PLoS One, 7, e40677.
258. Thakur, A. et al. (2013) Immunotherapy and immune evasion in prostate cancer. Cancers (Basel)., 5, 569–590.
259. Kryczek, I. et al. (2009) Phenotype, distribution, generation, and functional and clinical relevance of Th17 cells in the human tumor environments. Blood, 114, 1141–1149.
260. Lee, Y. et al. (2012) Protumoral role of monocytes in human B-cell precursor acute lymphoblastic leukemia: involvement of the chemokine CXCL10. Blood, 119, 227–237.
261. Martins, V.L. et al. (2009) Increased invasive behaviour in cutaneous squamous cell carcinoma with loss of basement-membrane type VII collagen. J. Cell Sci., 122(Pt 11), 1788–1799.
262. Jiang, Z. et al. (2010) CXCL10 expression and prognostic significance in stage II and III colorectal cancer. Mol. Biol. Rep., 37, 3029–3036.
263. Kawada, K. et al. (2004) Pivotal role of CXCR3 in melanoma cell metastasis to lymph nodes. Cancer Res., 64, 4010–4017.
264. Tannenbaum, C.S. et al. (1998) The CXC chemokines IP-10 and Mig are necessary for IL-12-mediated regression of the mouse RENCA tumor. J. Immunol., 161, 927–932.
265. Walser, T.C. et al. (2006) Antagonism of CXCR3 inhibits lung metastasis in a murine model of metastatic breast cancer. Cancer Res., 66, 7.
266. Ling, C.C. et al. (2014) Post-transplant endothelial progenitor cell mobilization via CXCL10/CXCR3 signaling promotes liver tumor growth. J. Hepatol., 60, 103–109.
267. Billottet, C. et al. (2013) CXCR3, a double-edged sword in tumor progression and angiogenesis. Biochim. Biophys. Acta, 1836, 287–295.
268. Nakayama, K. (2013) cAMP-response element-binding protein (CREB) and NF-κB transcription factors are activated during prolonged hypoxia and cooperatively regulate the induction of matrix metalloproteinase MMP1. J. Biol. Chem., 288, 22584–22595.
269. Radisky, D.C. et al. (2006) Matrix metalloproteinase-induced genomic instability. Curr. Opin. Genet. Dev., 16, 45–50.
270. Urtasun, R. et al. (2009) Reactive nitrogen species switch on early extracellular matrix remodeling via induction of MMP1 and TNFalpha. Gastroenterology, 136, 1410–22, e1.
271. Nutt, J.E. et al. (1998) Matrix metalloproteinase-1 is induced by epidermal growth factor in human bladder tumour cell lines and is detectable in urine of patients with bladder tumours. Br. J. Cancer, 78, 215–220.
272. Pritchard, S.C. et al. (2001) Expression of matrix metalloproteinases 1, 2, 9 and their tissue inhibitors in stage II non-small cell lung cancer: implications for MMP inhibition therapy. Oncol. Rep., 8, 421–424.
273. Lu, X. et al. (2009) ADAMTS1 and MMP1 proteolytically engage EGF-like ligands in an osteolytic signaling cascade for bone metastasis. Genes Dev., 23, 13.
274. Bao, W. et al. (2010) HER2-mediated upregulation of MMP-1 is involved in gastric cancer cell invasion. Arch. Biochem. Biophys., 499, 49–55.
275. Garamszegi, N. et al. (2012) Matrix metalloproteinase-1 contribution to sarcoma cell invasion. J. Cell. Mol. Med., 16, 1331–1341.
276. Liao, M. et al. (2012) Prognostic value of matrix metalloproteinase-1/proteinase-activated receptor-1 signaling axis in hepatocellular carcinoma. Pathol. Oncol. Res., 18, 7.
277. Fanjul-Fernández, M. et al. (2013) Matrix metalloproteinase Mmp-1a is dispensable for normal growth and fertility in mice and promotes lung cancer progression by modulating inflammatory responses. J. Biol. Chem., 288, 14647–14656.
278. Al-Hassan, N.N. et al. (2012) Differential roles of uPAR in peritoneal ovarian carcinomatosis. Neoplasia, 14, 259–270.
279. Reyes-Hernández, O.D. et al. (2009) Aromatic hydrocarbons upregulate glyceraldehyde-3-phosphate dehydrogenase and induce changes in actin cytoskeleton. Role of the aryl hydrocarbon receptor (AhR). Toxicology, 266, 30–37.
280. Marlowe, J.L. et al. (2005) Aryl hydrocarbon receptor, cell cycle regulation, toxicity, and tumorigenesis. J. Cell. Biochem., 96, 1174–1184.

281. Korkalainen, M. et al. (2012) induces genomic instability in mouse embryonic fibroblasts. PLoS One, 7, e37895.
282. Caruso, J.A. et al. (2006) Aryl hydrocarbon receptor modulation of tumor necrosis factor-alpha-induced apoptosis and lysosomal disruption in a hepatoma model that is caspase-8-independent. J. Biol. Chem., 281, 10954–10967.
283. Park, K.T. et al. (2005) The aryl hydrocarbon receptor predisposes hepatocytes to Fas-mediated apoptosis. Mol. Pharmacol., 67, 612–622.
284. Shervington, A. et al. (2007) Identification of a novel co-transcription of P450/1A1 with telomerase in A549. Genes Dev., 388, 7.
285. Sarkar, P. et al. (2006) Activation of telomerase in BeWo cells by estrogen and 2,3,7,8-tetrachlorodibenzo-p-dioxin in co-operation with c-Myc. I. Int. J. Oncol., 28, 9.
286. Ray, S. et al. (2009) Activation of the aryl hydrocarbon receptor by TCDD inhibits senescence: a tumor promoting event. Biochem. Pharmacol., 77, 8.
287. Lahoti, T.S. et al. (2014) Aryl hydrocarbon receptor antagonism attenuates growth factor expression, proliferation, and migration in fibroblast-like synoviocytes from patients with rheumatoid arthritis. J. Pharmacol. Exp. Ther., 348, 236–245.
288. Budinsky, R.A. et al. (2014) Mode of action and dose-response framework analysis for receptor-mediated toxicity: The aryl hydrocarbon receptor as a case study. Crit. Rev. Toxicol., 44, 83–119.
289. Goode, G.D. et al. (2013) Knockdown of aberrantly upregulated aryl hydrocarbon receptor reduces tumor growth and metastasis of MDA-MB-231 human breast cancer cell line. Int. J. Cancer, 133, 2769–2780.
290. Pierre, S. et al. (2014) Aryl hydrocarbon receptor-dependent induction of liver fibrosis by dioxin. Toxicol. Sci., 137, 11.
291. Opitz, C.A. et al. (2011) An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. Nature, 478, 197–203.
292. Hosokawa, S. et al. (1993) Hormonal disregulation mechanism in the rat thyroid tumor induced by diniconazole. J. Toxicol. Sci., 18, 11.
293. Koç, H. et al. (2012) Severe hepatotoxicity and acute renal failure caused by diniconazole. Clin. Res. Hepatol. Gastroenterol., 36, e104–e105.
294. Wang, X.F. et al. (2006) Inhibitory effects of pesticides on proteasome activity: implication in Parkinson's disease. Neurobiol. Dis., 23, 198–205.
295. Gopinath, S. et al. (2011) Mechanism of p27 upregulation induced by downregulation of cathepsin B and uPAR in glioma. Mol. Oncol., 5, 12.
296. Taylor, T.R. et al. (2011) Ziram activates mitogen-activated protein kinases and decreases cytolytic protein levels in human natural killer cells. Toxicol. Mech. Methods., 21, 8.
297. Scarabelli, L. et al. (1993) Relationship between poly(ADP-ribose) polymerase activity and DNA damage induced by zinc dithiocarbamates in mouse and rat liver. Mutat. Res., 302, 1–6.
298. Li, Q. et al. (2012) Mechanism of ziram-induced apoptosis in human natural killer cells. Int. J. Immunopathol. Pharmacol., 25, 883–891.
299. Coussens, L.M. et al. (2013) Neutralizing tumor-promoting chronic inflammation: a magic bullet? Science, 339, 286–291.
300. Blog, D.N. (2012) Take action! New farm bill amendments attack your health and environment; beyond pesticides. Daily News Blog.
301. Cima, F. et al. (2008) Toxic effects of new antifouling compounds on tunicate haemocytes I. Sea-nine 211 and chlorothalonil. Aquat. Toxicol., 86, 299–312.
302. Pariseau, J. et al. (2011) Effects of pesticide compounds (chlorothalonil and mancozeb) and benzo[a]pyrene mixture on aryl hydrocarbon receptor, p53 and ubiquitin gene expression levels in haemocytes of soft-shell clams (Mya arenaria). Ecotoxicology, 20, 1765–1772.
303. Lebailly, P. et al. (1997) Assessment of DNA damage induced *in vitro* by etoposide and two fungicides (carbendazim and chlorothalonil) in human lymphocytes with the comet assay. Mutat. Res., 375, 205–217.
304. Greenlee, A.R. et al. (2004) Low-dose agrochemicals and lawn-care pesticides induce developmental toxicity in murine preimplantation embryos. Environ. Health Perspect., 112, 7.
305. Tessier, D.M. et al. (2001) Increased ErbB-2 tyrosine kinase activity, MAPK phosphorylation, and cell proliferation in the prostate cancer cell line LNCaP following treatment by select pesticides. Toxicol. Sci., 60, 6.
306. Wilkinson, C.F. et al. (1996) A mechanistic interpretation of the oncogenicity of chlorothalonil in rodents and an assessment of human relevance. Regul. Toxicol. Pharmacol., 24(1 Pt 1), 69–84.
307. Rencüzoğullari, E. et al. (2008) The effects of food protector biphenyl on sister chromatid exchange, chromosome aberrations, and micronucleus in human lymphocytes. Drug Chem. Toxicol., 31, 263–274.
308. Park, J.B. et al. (2012) Magnolol-induced apoptosis in HCT-116 colon cancer cells is associated with the AMP-activated protein kinase signaling pathway. Biol. Pharm. Bull., 35, 1614–1620.
309. Pisano, M. et al. (2007) Antiproliferative and pro-apoptotic activity of eugenol-related biphenyls on malignant melanoma cells. Mol. Cancer Ther., 6, 3.
310. Holland, J.P. et al. (2013) Synthesis and evaluation of biphenyl compounds as kinesin spindle protein inhibitors. Chem. Biodivers., 10, 18.
311. Brown, J.F. Jr et al. (2007) Polychlorinated biphenyls modulated tumorigenesis in Sprague Dawley rats: correlation with mixed function oxidase activities and superoxide (O2*) formation potentials and implied mode of action. Toxicol. Sci., 98(2):375–394.
312. Nozaki, S. et al. (2003) Activity of biphenyl matrix metalloproteinase inhibitor BAY 12–9566 in a human breast cancer orthotopic model. Clin. Exp. Metastasis., 20, 6.
313. Shin, K.D. et al. (2005) Blocking tumor cell migration and invasion with biphenyl isoxazole derivative KRIBB3, a synthetic molecule that inhibits Hsp27 phosphorylation. J. Biol. Chem., 280, 10.
314. Hwang, E.S. et al. (2010) Magnolol suppresses metastasis via inhibition of invasion, migration, and matrix metalloproteinase-2/-9 activities in PC-3 human prostate carcinoma cells. Biosci. Biotechnol. Biochem., 74, 961–967.
315. Bunaciu, R.P. et al. (2007) The effect of dietary glycine on the hepatic tumor promoting activity of polychlorinated biphenyls (PCBs) in rats. Toxicology, 239, 147–155.
316. Yamada, S. et al. (2013) AMP-activated protein kinase-mediated glucose transport as a novel target of tributyltin in human embryonic carcinoma cells. Metallomics, 5, 484–491.
317. Isomura, M. et al. (2013) Tributyltin-induced endoplasmic reticulum stress and its Ca(2+)-mediated mechanism. Toxicol. Appl. Pharmacol., 272, 10.
318. Kirchner, S. et al. (2010) Prenatal exposure to the environmental obesogen tributyltin predisposes multipotent stem cells to become adipocytes. Mol. Endocrinol., 24, 526–539.
319. Tsunoda, M. et al. (2006) Subacute administration of tributyltin chloride modulates neurotransmitters and their metabolites in discrete brain regions of maternal mice and their F1 offspring. Toxicol. Ind. Health, 22, 15–25.
320. Tiano, L. et al. (2001) DNA damage induced by organotins on trout-nucleated erythrocytes. Appl. Organometal. Chem., 15, 6.
321. Mitra, S. et al. (2013) Tributyltin chloride induced testicular toxicity by JNK and p38 activation, redox imbalance and cell death in sertoli-germ cell co-culture. Toxicology, 314, 12.
322. Zhang, J. et al. (2011) Tributyltin chloride results in dorsal curvature in embryo development of Sebastiscus marmoratus via apoptosis pathway. Chemosphere, 82, 437–442.
323. Nakatsu, Y. et al. (2007) Concentration dependence of the mechanisms of tributyltin-induced apoptosis. Toxicol. Sci., 97, 438–447.
324. Tiano, L. et al. (2003) Effect of tributyltin on trout blood cells: changes in mitochondrial morphology and functionality. Biochim. Biophys. Acta., 1640, 8.
325. Ishihara, Y. et al. (2012) Tributyltin induces oxidative stress and neuronal injury by inhibiting glutathione S-transferase in rat organotypic hippocampal slice cultures. Neurochem. Int., 60, 782–790.
326. Singh, T. et al. (2006) Microscopic, biochemical and physiological assessment of the effect of methylene bisthiocyanate on the sapstain fungus ophiostoma floccosum. Eur. J. Plant Pathol., 114, 12.
327. Thejass, P. et al. (2006) Antimetastatic activity of sulforaphane. Life Sci., 78, 8.
328. Jee, H.G. et al. (2011) Sulforaphane inhibits oral carcinoma cell migration and invasion in vitro. Phytother. Res., 25, 6.
329. Xu, T. et al. (2012) Dual roles of sulforaphane in cancer treatment. Anticancer Agents Med. Chem., 12, 12.

330. Burka, L.T. (1993) NTP technical report on the toxicity studies of Methylene Bis(thiocyanate) (CAS No. 6317-18-6) Administered by Gavage to F344/N Rats and B6C3F1 Mice. Toxic. Rep. Ser., 32, 1–E7.
331. Bulger, W.H. et al. (1978) Interactions of methoxychlor, methoxychlor base-soluble contaminant, and 2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane with rat uterine estrogen receptor. J. Toxicol. Environ. Health, 4, 881–893.
332. Skinner, M.K. et al. (2011) Epigenetic transgenerational actions of endocrine disruptors. Reprod. Toxicol., 31, 337–343.
333. Symonds, D.A. et al. (2005) Methoxychlor induces proliferation of the mouse ovarian surface epithelium. Toxicol. Sci., 83, 8.
334. Hall, J.M. et al. (2013) Endocrine disrupting chemicals promote the growth of ovarian cancer cells via the ER-CXCL12-CXCR4 signaling axis. Mol. Carcinog., 52, 715–725.
335. Shishodia, S. et al. (2005) N-(4-hydroxyphenyl)retinamide inhibits invasion, suppresses osteoclastogenesis, and potentiates apoptosis through down-regulation of I(kappa)B(alpha) kinase and nuclear factor-kappaB-regulated gene products. Cancer Res., 65, 9555–9565.
336. Dong, G.H. et al. (2012) Induction of p53-mediated apoptosis in splenocytes and thymocytes of C57BL/6 mice exposed to perfluorooctane sulfonate (PFOS). Toxicol. Appl. Pharmacol., 264, 292–299.
337. Eriksen, K.T. et al. (2010) Genotoxic potential of the perfluorinated chemicals PFOA, PFOS, PFBS, PFNA and PFHxA in human HepG2 cells. Mutat. Res., 700, 39–43.
338. Wang, X. et al. (2013) PFOS-induced apoptosis through mitochondrion-dependent pathway in human-hamster hybrid cells. Mutat. Res., 754, 51–57.
339. Lee, Y.J. et al. (2013) Perfluorooctane sulfonate-induced apoptosis of cerebellar granule cells is mediated by ERK ½ pathway. Chemosphere, 90, 1597–1602.
340. Elcombe, C.R. et al. (2012) Hepatocellular hypertrophy and cell proliferation in Sprague-Dawley rats from dietary exposure to potassium perfluorooctanesulfonate results from increased expression of xenosensor nuclear receptors PPARα and CAR/PXR. Toxicology, 293, 16–29.
341. Fair, P.A. et al. (2011) Effects of environmentally-relevant levels of perfluorooctane sulfonate on clinical parameters and immunological functions in B6C3F1 mice. J. Immunotoxicol., 8, 17–29.
342. Liao, Y. et al. (2012) Evaluation of cellular response to perfluorooctane sulfonate in human umbilical vein endothelial cells. Toxicol. In Vitro, 26, 421–428.
343. Betancourt, A.M. et al. (2012) Altered carcinogenesis and proteome in mammary glands of rats after prepubertal exposures to the hormonally active chemicals bisphenol a and genistein. J. Nutr., 142, 1382S–1388S.
344. Fillon, M. (2012) Getting it right: BPA and the difficulty proving environmental cancer risks. J. Natl. Cancer Inst., 104, 652–655.
345. Lee, S. et al. (2013) Neurotoxic effects of bisphenol AF on calcium-induced ROS and MAPKs. Neurotox. Res., 23, 249–259.
346. Pupo, M. et al. (2012) Bisphenol A induces gene expression changes and proliferative effects through GPER in breast cancer cells and cancer-associated fibroblasts. Environ. Health Perspect., 120, 1177–1182.
347. Zhu, H. et al. (2010) Environmental endocrine disruptors promote invasion and metastasis of SK-N-SH human neuroblastoma cells. Oncol. Rep., 23, 129–139.
348. Jenkins, S. et al. (2011) Chronic oral exposure to bisphenol A results in a nonmonotonic dose response in mammary carcinogenesis and metastasis in MMTV-erbB2 mice. Environ. Health Perspect., 119, 1604–1609.
349. Derouiche, S. et al. (2013) Bisphenol A stimulates human prostate cancer cell migration via remodelling of calcium signalling. Springerplus, 2, 54.
350. Feng, Y. et al. (2012) Bisphenol AF may cause testosterone reduction by directly affecting testis function in adult male rats. Toxicol. Lett., 211, 201–209.
351. Pitot, H.C. et al. (1989) Regulation of the expression of some genes for enzymes of glutathione metabolism in hepatotoxicity and hepatocarcinogenesis. Toxicol. Appl. Pharmacol., 97, 23–34.
352. Stiborová, M. et al. (2002) Sudan I is a potential carcinogen for humans: evidence for its metabolic activation and detoxication by human recombinant cytochrome P450 1A1 and liver microsomes. Cancer Res., 62, 5678–5684.
353. Stiborová, M. et al. (2009) Oxidation of the carcinogenic non-aminoazo dye 1-phenylazo-2-hydroxy-naphthalene (Sudan I) by cytochromes P450 and peroxidases: a comparative study. Interdiscip. Toxicol., 2, 195–200.
354. Ohno, M. et al. (2012) Sudan III dye strongly induces CYP1A1 mRNA expression in HepG2 cells. J. Biochem. Mol. Toxicol., 26, 7.
355. Elliott, B.M. et al. (1997) CI solvent yellow 14 shows activity in the bone marrow micronucleus assay in both the rat and mouse. Mutagenesis, 12, 255–258.
356. Pitot, H.C. et al. (1989) Critical parameters in the quantitation of the stages of initiation, promotion, and progression in one model of hepatocarcinogenesis in the rat. Toxicol. Pathol., 17(4 Pt 1), 594–611; discussion 611.
357. Gough, W. et al. (2011) A quantitative, facile, and high-throughput image-based cell migration method is a robust alternative to the scratch assay. J. Biomol. Screen., 16, 155–63.
358. Wang, L. et al. (2008) An automatic and quantitative on-chip cell migration assay using self-assembled monolayers combined with real-time cellular impedance sensing. Lab Chip, 8, 872–878.
359. Nie, F.Q. et al. (2007) On-chip cell migration assay using microfluidic channels. Biomaterials, 28, 4017–4022.
360. Moody, M.D. et al. (2001) Array-based ELISAs for high-throughput analysis of human cytokines. Biotechniques, 31, 186–190, 192.
361. Xia, M. et al. (2009) Identification of small molecule compounds that inhibit the HIF-1 signaling pathway. Mol. Cancer, 8, 117.
362. Hellweg, C.E. et al. (2003) Generation of stably transfected mammalian cell lines as fluorescent screening assay for NF-kappaB activation-dependent gene expression. J. Biomol. Screen., 8, 511–521.
363. Huang, H. et al. (2012) High-throughput screening for bioactive molecules using primary cell culture of transgenic zebrafish embryos. Cell Rep., 2, 695–704.
364. Kiss-Toth, E. et al. (2000) A novel mammalian expression screen exploiting green fluorescent protein-based transcription detection in single cells. J. Immunol. Methods, 239, 125–135.
365. Rapisarda, A. et al. (2002) Identification of small molecule inhibitors of hypoxia-inducible factor 1 transcriptional activation pathway. Cancer Res., 62, 4316–4324.
366. Woldemichael, G.M. et al. (2006) Development of a cell-based reporter assay for screening of inhibitors of hypoxia-inducible factor 2-induced gene expression. J. Biomol. Screen., 11, 678–687.
367. Zerwes, H.G. et al. (2002) A multiparameter screening assay to assess the cytokine-induced expression of endothelial cell adhesion molecules. Anal. Biochem., 304, 166–173.