Toxicology and Applied Pharmacology 283 (2015) 83–91

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Topological, functional, and dynamic properties of the protein interaction networks rewired by benzo(a)pyrene

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ARTICLE INFO

Article history:
Received 18 September 2014
Revised 19 December 2014
Accepted 6 January 2015
Available online 14 January 2015

Keywords:
Benzo(a)pyrene
Protein network
Module
Signaling pathway

ABSTRACT

Benzo(a)pyrene is a common environmental and foodborne pollutant that has been identified as a human carcinogen. Although the carcinogenicity of benzo(a)pyrene has been extensively reported, its precise molecular mechanisms and the influence on system-level protein networks are not well understood. To investigate the system-level influence of benzo(a)pyrene on protein interactions and regulatory networks, a benzo(a)pyrene-rewired protein interaction network was constructed based on 769 key proteins derived from more than 500 literature reports. The protein interaction network rewired by benzo(a)pyrene was a scale-free, highly-connected biological system. Ten modules were identified, and 25 signaling pathways were enriched, most of which belong to the human diseases category, especially cancer and infectious disease. In addition, two lung-specific and two liver-specific pathways were identified. Three pathways were specific in short and medium-term networks (<48 h), and five pathways were enriched only in the medium-term network (6 h–48 h). Finally, the expression of linker genes in the network was validated by Western blotting. These findings establish the overall, tissue- and time-specific benzo(a)pyrene-rewired protein interaction networks and provide insights into the biological effects and molecular mechanisms of action of benzo(a)pyrene.

Introduction

Benzo(a)pyrene, the most studied member of the polycyclic aromatic hydrocarbons (PAHs), has been used as a marker of exposure to carcinogenic PAHs (Benford et al., 2010). This ubiquitous environmental carcinogen is formed from the incomplete combustion of organic materials and is commonly found with other PAHs in tobacco smoke, charcoal-grilled meat, and PAH-contaminated soil (Gelboin, 1980; Phillips, 1999). Benzo(a)pyrene is metabolized in the body to form various reactive metabolites, which may elicit toxicity through binding covalently to cellular elements such as DNA and through generating reactive oxygen species. Benzo(a)pyrene, which requires metabolic activation to become carcinogenic (Rubin, 2001), has been classified as a human Group 1 carcinogen by the International Agency for Research on Cancer (IARC) (Einem Lindeman et al., 2011). Due to the adverse biological effects of benzo(a)pyrene, including carcinogenicity, teratogenicity, neurotoxicity, and immunotoxicity, it has been used as a model compound for many toxicological investigations (Verma et al., 2012).

Modern toxicology encourages a transformation of toxicity testing from animal studies to pathway-based approaches using human-relevant cells (Gibb, 2008; Bhattacharya et al., 2011; Adeleye et al., 2014). Cells represent complex homeostasis systems with large numbers of molecules, including proteins, carbohydrates, and lipids, that are essential for growth and normal function (Said et al., 2004). Environmental pollutants such as benzo(a)pyrene could disrupt the homeostasis through individual molecules, and thereby exert their toxicological effects. Therefore, it is important in toxicology research to understand the structure and dynamics of the interaction network rewired by environmental pollutants (Barabasi and Oltvai, 2004). Although the phenotypic toxicity effects of benzo(a)pyrene in experimental animals has been elucidated, how benzo(a)pyrene induces molecular disturbances in the overall protein interaction network and the dynamic characteristics of this effect in different tissues and exposure times remain unclear.

To explore the influence of benzo(a)pyrene on intracellular protein interaction networks, bioinformatics methods and network-based analyses were employed to investigate the molecular behavior from a holistic perspective. The protein interaction networks rewired by benzo(a)pyrene were mapped, and their topological and tissue/time dynamic properties were clarified. Through modularity analysis, key

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http://dx.doi.org/10.1016/j.taap.2015.01.006
0041-008X/

signaling pathways were found to be involved in the toxicity of benzo(a)pyrene, thus providing a broad understanding of the mechanisms of action of benzo(a)pyrene in cancer formation.

Materials and methods

Database. The STRING database (version 9.05, http://string-db.org), which is based on the known and predicted protein–protein interactions derived from sources of genomic context, high-throughput experiments, conserved co-expression and previous knowledge, was used (Szklarczyk et al., 2011). Through searching the gene symbols, data about the interactions for each protein were obtained. Only proteins that were experimentally confirmed to be influenced by benzo(a)pyrene were chosen to create a graph based on physical interactions and functional associations. By use of the STRING database, the individual interaction networks for each selected protein were built

Network construction and simplification. Benzo(a)pyrene-rewired protein interaction networks were constructed and simplified as described previously (Huang et al., 2013). The individual interaction networks for each protein were selected according to STRING-computed confidence scores. The Cytoscape software (version 2.8.3; http://www.cytoscape.org/), with a variety of network-related plugins, supports a network visualization and integration platform (Smoot et al., 2011). By use of Cytoscape software, the individual protein networks with confidence scores ranging in the top interval (most between 0.9 and 1.0) were chosen to construct the full protein interaction network rewired by benzo(a)pyrene.

For the total protein interaction network rewired by benzo(a)pyrene, according to the GO term of each protein in the Biological Process of the Gene Ontology (GO), all individual protein networks were merged into six sub-networks corresponding to different aspects of cancer (apoptosis, angiogenesis, drug response, inflammatory response, migration, and oxidative stress). The sub-networks were further integrated into the full protein interaction network with the plugin, Advanced Network Merge (Version 1.16). For tissue/time-dependent benzo(a)pyrene networks, the altered proteins in various experimental settings were first selected and then the specific networks were constructed as described above.

In all networks, there were two types of proteins: 1) key proteins that were selected from literature reports and 2) linker proteins that were overlapping proteins between the individual interaction networks of key proteins. To reduce noise, the networks were simplified according the following principles: 1) proteins that were neither key proteins nor linker proteins were deleted; 2) linker proteins that linked other linker proteins were deleted; and 3) if two key proteins were linked by more than one linker protein, the linker proteins with fewer connections in the network were deleted.

Network topological analysis. In the process of network analysis, Cytoscape software and the Network Analyzer plugin (version 1.0, http://med.bioinf.mpi-inf.mpg.de/netanalyzer/) were used to calculate the basic topological parameters of the benzo(a)pyrene-rewired network, i.e. degree distribution, degree exponent, shortest path length distribution, and the average clustering coefficient distribution. To validate the clustering coefficient of the total protein interaction network, 100 randomized networks were generated by rewiring edges, while preserving the degrees of notes in the original network, and their clustering coefficients were obtained using the Cytoscape Random Network plugin (version 1.5, http://sites.google.com/site/randomnetworkplugin/).

Network modular analysis and pathway enrichment. With the plugin MCODE (version 1.2, http://baderlab.org/Software/MCODE), the modules from the total protein interaction network rewired by benzo(a)pyrene were identified (Bader and Hogue, 2003). Each module was scored by Cytoscape software through the density and size; a higher score meant a tighter module. The threshold score was set at 1.5. Within the modules, pathway analyses were processed by the ClueGO plugin (version 1.4) according to the Kyoto Encyclopedia of Genes and Genomes database (KEGG, 2012.10.22) (Bindea et al., 2009). Only KEGG terms with the overview "true" were considered as positive.

Comparison of multiple networks. Pathway comparisons among various tissue/time specific networks were accomplished using the ClueGO plugin (version 1.4) in Cytoscape according to the KEGG database, which analyzed pathways through the perspective of

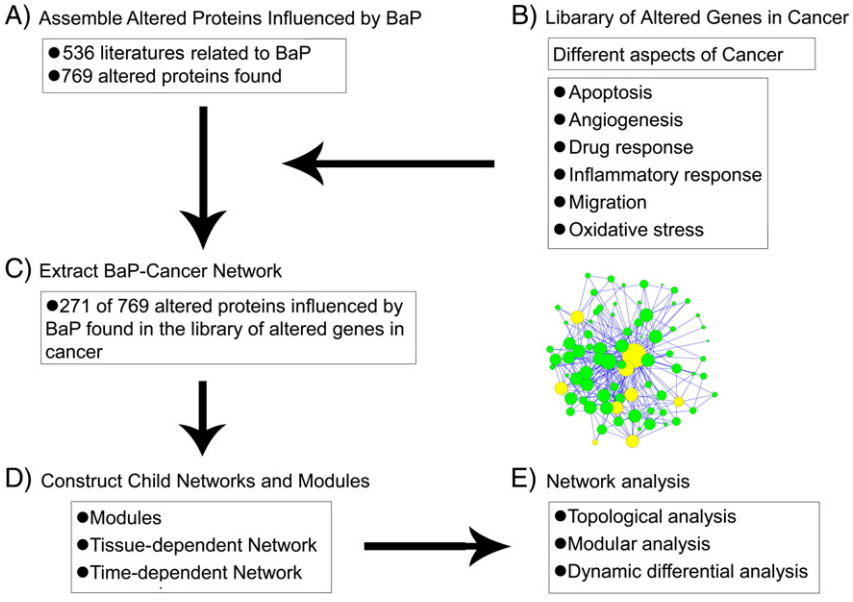

Fig. 1. Schematic diagram of analyses. (A–C) The experimentally determined altered proteins impacted by benzo(a)pyrene were extracted (A), classified into different aspects of cancer according to the GO database (B), and integrated into the total protein interaction network associated with the effects of benzo(a)pyrene on cancer cells (C). (D) The total network was partitioned into different modules, and tissue/time specific networks were constructed in the same way. (E) The networks were subjected to topological, modular and dynamic analyses. Finally, key pathways were enriched and validated.