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Development and characterization of monoclonal antibodies against human aryl hydrocarbon receptor

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ABSTRACT

Aryl hydrocarbon receptor (AhR), a ligand-dependent nuclear receptor, is involved in a diverse spectrum of biological and toxicological effects. Due to the lack of three dimensional (3D) crystal or nuclear magnetic resonance structure, the mechanisms of these complex effects of AhR remain to be unclear. Also, commercial monoclonal antibodies (mAbs) against human AhR protein (hAhR), as alternative immunological tools, are very limited. Thus, in order to provide more tools for further studies on hAhR, we prepared two mAbs (1D6 and 4A6) against hAhR. The two newly generated mAbs specifically bound to amino acids 484–508 (located in transcription activation domain) and amino acids 201–215 (located in Per-ARNT-Sim domain) of hAhR, respectively. These epitopes were new as compared with those of commercial mAbs. The mAbs were also characterized by enzyme-linked immunosorbent assay, western blot, immunoprecipitation and indirect immunofluorescence assay in different cell lines. The results showed that the two mAbs could recognize the linearized AhRs in six different human cell lines and a rat hepatoma cell line, as well as the hAhR with native conformations. We concluded that the newly generated mAbs could be employed in AhR-based bioassays for analysis of environmental contaminants, and held great potential for further revealing the spatial structure of AhR and its biological functions in future studies.

Introduction

The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor which belongs to the basic helix-loop-helix (bHLH)-Per-ARNT-Sim (PAS) family (Burbach et al., 1992). Functional domains of AhR are classified to a bHLH region, two PAS domains (A and B) and a transcription activation domain (TAD). The aryl hydrocarbon receptor can be activated by exogenous chemicals, such as halogenated aromatic hydrocarbons, non-halogenated polycyclic aromatic hydrocarbons, and other dioxin-like chemicals (DLCs), as well as endogenous compounds, such as 6-formylindolo-[3,2-b]-carbazole, bilirubin and lipoxinA4 (Denison and Nagy, 2003; Nguyen and Bradfield, 2007). The activation of AhR by these compounds leads to regulation of the expression of a battery of genes resulting in diverse biological and toxicological effects, including dermal, hepatic, cardiac and immunotoxic response, wasting syndrome, reproductive and developmental toxicities (Beischlag et al., 2008; Flaveny et al., 2010; Birnbaum and Tuomisto, 2000; Bock, 1994).

Apart from the presence of endogenous ligands, studies using AhR knockout (KO) mice further indicated that AhR also played roles in normal physiology and pathology. It has been reported

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that almost half of the AhR-deficient mice died shortly after birth. Although the survivor reached maturity and were able to be fertile, they showed decreased accumulation of lymphocytes in the spleen and lymph nodes (Fernandez-Salguero et al., 1995). In the immune system, AhR was involved in regulating the antigen responses in Th17 cells, dendritic cells, and other adaptive immune cells (Esser, 2009; Esser et al., 2009; Tian et al., 2015). In addition, smaller liver size and hepatic deformation were observed in adult AhR KO mice (Harstad et al., 2006), and the animals were more likely to develop liver tumors after exposure to diethylnitrosamine (Fan et al., 2010). Besides, a recent investigation demonstrated that AhR KO mice had a higher risk of developing bladder stones (Butler et al., 2012). However, the mechanisms for AhR being involved in these physiological and pathological responses were not well-understood.

Given the biological and toxicological importance of AhR exhibited via interactions with various exogenous and endogenous compounds of diverse chemical structures, the mysterious molecular events upon its ligand bindings have been attracting increasing attentions. Due to the lack of 3D crystal or nuclear magnetic resonance structure of AhR, monoclonal antibodies (mAbs), binding specifically to various functional domains of AhR, could serve as effective tools for sophisticated studies on the molecular events and mechanisms. Apart from that, mAbs can also be used to detect AhR ligands in the environment, such as in Ah-Immunoassay (Ah-I) (Tian et al., 2012).

Currently, the commercial anti-hAhR mAbs are specifically raised against the synthetic peptide RKRRKPVKPIPAEGIK (amino acid (a.a.) 12–17 & a.a. 22–31) or recombinant proteins corresponding to a.a. 721–821, 637–848 or 1–848 of hAhR. Given the homology of hAhR with murine AhR, functional domains of hAhR could be predicted based on those of mouse AhR (mAhR, Fukunaga et al., 1995; Whitelaw et al., 1994), including the PAS B domain (a.a. 230–397) of mAhR for exogenous ligand binding, the bHLH region (a.a. 27–79) and the region encompassing the PAS B repeat (a.a. 182–374) for Hsp 90 binding, a part of bHLH region (a.a. 27–39) for DNA binding, regions encompassing a.a. 40–79 and a.a. 121–289 for dimerization and the Q-rich region (a.a. 490 to 805) in the TAD domain for transcription activation. Thus, the epitopes of the commercial mAbs might specifically bind to the peptides located in only two function regions of hAhR, bHLH and Q-rich domains, which is insufficient to study the complex molecular events upon activation of hAhR involving different functional domains. Therefore, in the present study, we employed a recombinant protein of full-length hAhR expressed in *Escherichia coli* BL21 cells aiming to generate new mAbs with novel epitopes belonging to different functional domains of hAhR. Two new mAbs were selected and subjected to series characterizations to reveal the recognition ability, the epitopes and potential applications.

1. Materials and methods

1.1. Animals and cell culture

All mice care and experimentation were approved by the Committee of Animal Care at the Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences (RCEES). Female BALB/c mice, six weeks of age, were purchased from Vital River Laboratories (Beijing, China). The mice were fed on a standard laboratory chow diet, housed four or three per cage, maintained at (20 ± 1)°C, relative humidity of 50% ± 10%, with a 12 hr/12 hr light/dark cycle and sacrificed via CO_2 asphyxiation.

Two hepatoma cell lines HepG2 (human) and H4IIE (rat) were kind gifts from Dr. Michael S. Denison (University of California, Davis, CA, USA). Mouse skeletal muscle cell line (C2C12) was from Dr. Karl WK Tsim (The Hong Kong University of Science and Technology, Hong Kong, China). Human renal tubular epithelial cell line (HKC) was from Dr. Wei Liu (Peking University Hospital, Beijing, China). Human cervical epithelial cell line (HeLa) was from Dr. Benzhan Zhu (RCEES, Beijing, China). Human embryonic kidney 293 T (HEK 293 T) cell line, human breast cancer cell line (MCF-7), human neuroblastoma cell line (SK-N-SH) and mouse myeloma cell line (SP2/0) were obtained from the cell resource center of Chinese Academy of Medical Sciences (Beijing, China). The hepatoma cell lines were cultured in α-MEM (alpha Eagle's minimal essential medium). MCF-7 cells, HeLa cells, HEK293T cells, C2C12 cells and SK-N-SH cells were grown in DMEM (Dulbecco's Modified Eagle Medium). HKC cells were cultured in DMEM/F12 medium. SP2/0 cells and hybridoma cells were cultured in RPMI 1640 medium. All cell culture medium were supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin–Streptomycin Solution (P/S). The cell lines were seeded in 100 mm dishes or 96-well plates and incubated at 37°C under a 5% CO_2, 95% humidity environment. All reagents for cell culture were purchased from Invitrogen (CA, USA).

1.2. Preparation and characterization of mAbs against hAhR

The prokaryotic expressing plasmid pGEX4T-1/GST-hAhR was constructed using primers of 5′-AAC AGT CGA CTC ATG AAC AGC AGC AGC GCC AA-3′ and 5′-AAA AGC GGC CGC TAC AGG AAT CCA CTG GAT GTC A-3′ with Sal I and Not I sites (underlined), respectively. The hAhR sequence (Accession: NM_001621.4) was inserted. Glutathione S-transferase (GST)-tagged hAhR fusion proteins (GST-hAhRs), and GST proteins were overexpressed in *E. coli* BL21 by transformation of plasmids, pGEX4T-1/GST-hAhR and pGEX4T-1/GST, respectively. The GST-hAhRs were present in the inclusion bodies. Mixed proteins at around 110 kDa were obtained from the inclusion bodies which were separated on 6% SDS-PAGE. The GST proteins, used as control, were purified on glutathione-agarose beads. Seven adult female BALB/c mice were immunized GST-hAhRs (20 μg per mouse) for 4 times by subcutaneous injection. Subsequently, according to the standard methodology (Köhler and Milstein, 1976), spleen cells from three selected immunized mice were fused with SP2/0 cells in polyethylene glycol (PEG1500, Merck, Germany), and the hybridoma cells were cultivated.

The individual cultures were subjected to a screening test for their content of specific antibodies against GST-hAhRs but not GST proteins by standard indirect enzyme-linked immunosorbent assay (ELISA) with slight modifications. The 96-well ELISA plates were firstly incubated with either purified GST-hAhRs or GST in 0.1 mol/L Na_2CO_3 (pH = 9.2) overnight at 4°C at a concentration of 0.1 μg/well. After washing, 150 μL of 5% BSA was added to each well for 1 hr at 37°C. The coated plates were