Mitophagy induction through mito-CYP1B1/MnSOD axis facilitates Benzo[a]pyrene-mediated cell death

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Abstract

Background: Mitophagy, a special type of autophagy plays an important role in mitochondrial dynamics and mitochondria associated cell death. In this study, we examined the molecular mechanism of Benzo[a]pyrene (B[a]P) facilitated mitophagy-dependent cell death.

Method: Autophagic flux and mitophagy in HaCaT cells exposed to B[a]P were monitored by measuring levels and location of autophagy markers through Western blot, immunostaining and confocal microscopy. The mitochondrial energy status was quantified by oxygen consumption and flow cytometry. The involvement of MnSOD was evaluated by biochemical assay, docking and colocalization study.

Results: B[a]P found to display cytotoxicity in Beclin-1 deficient HaCaT cells indicating potential existence of non-apoptotic cell death with B[a]P. We showed that B[a]P triggered Beclin-1 dependent mitophagy through the mTOR-AMPK pathway. Intriguingly, mitophagy by B[a]P was suppressed in CYP1B1 and AhR knockdown HaCaT cells indicating the crucial role of B[a]P activation in mitophagy-regulated cell death. B[a]P was shown to accumulate dysfunction mitochondria resulting fall in ATP level along with abrogation of oxygen consumption rate in HaCaT cells. Importantly, supplementation of methyl pyruvate compensated B[a]P-induced fall in ATP level, mitigated reactive oxygen species burden and autophagy. Mechanistically, B[a]P inhibited MnSOD activity and further we found that activated mitochondrial CYP1B1 interact with MnSOD, inflicting mitophagy that leads to cell death.

Conclusions: Our study reveals an unknown aspect of B[a]P function by identifying it as a potential regulator of mitophagy dependent cell death, an important feature of B[a]P that may contribute to toxicity.

General significance: B[a]P-mediated mitophagy leads to apoptotic independent cell death to induce cellular toxicity.

Keywords: Benzo[a]pyrene, autophagic cell death, mitophagy, ATP depletion, CYP1B1, MnSOD
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Autophagy: The self degradation process
Autophagy: Health and disease

- Survival during short-term starvation
- Organelle turnover
- Protection against metabolic stress and DNA damage
- Clearance of aggregate-prone proteins
- Longevity
- Regulation of cell size
- Programmed cell death
- Defence against intracellular pathogens
- Antigen presentation
Autophagy dependent cell death

Bhutia SK et al, Advances in Cancer research, 2013
Air pollutants – Major problem of developing industrial cities like Rourkela

Particulate matter (PM) – forms a mixture of inorganic and organic component that vary in size, origin and composition:
- Coarse PM (2.5-10 µm)
- Fine PM (0.1 – 2.5 µm)
- Ultrafine PM (<0.1 µm)

Industrial PM contains organic volatile polycyclic aromatic hydrocarbon like Benzo[a]pyrene, anthracene, 1, 2-Benzpyrene, Dioxin, Dibenzo[!]furans.

Benzo[a]pyrene, Dioxin - most potent pollutant assessed by Environmental Protection Agency (EPA)

Major PM source: Industries, Power plant, Incinerators, Constructions

Cause: Increased mortality, morbidity including increased risk of cancer among industry workers
Benzo[a]pyrene, Dioxin- Group 1 carcinogen (IARC)

Cytochrome P450 inadvertently converts a precarcinogen like Benzo[a]pyrene and Dioxin- into highly potent carcinogens leading to widespread CANCER!
PAHs (Benzo[a]pyrene, Dioxin, etc)

Cytochrome p450

carcinogen

Genotoxic mechanism
- DNA adduct
- Reactive oxygen species
- Chromosomal breakage
- Activation of oncogenes
- Inactivation of tumor suppressor genes

Non-genotoxic mechanism
- Immune suppression/ activation
- Reactive oxygen species
- Epigenetic silencing/ activation
- Upregulation/activation of inflammatory mediators and signaling molecules (NF-kB, Cytokines, etc)

Genomic damage

Altered signal transduction

Mutation

Cancer

Das et al, Toxicology Molecular Methods
B[a]P induces autophagy

B[a]P induces autophagic cell death is independent of apoptosis
B[a]P induced autophagy follows AMPK/mTOR Pathway
B[a]P Activation

Das et al, Toxicology Molecular Methods
Selective types of Autophagy

- **Mitophagy**: Mitophagy refers to the autophagy of mitochondria.
- **Pexophagy**: Selective autophagy of peroxisomes.
- **Xenophagy**: The selective degradation of exogenous particles (e.g. Bacteria, Virus etc.)
- **Ribophagy**: Selective degradation of larger subunit of ribosome but not the smaller subunit.
- **Reticulophagy**: Selective degradation of Endoplasmic Reticulum (ER). It occurs when misfolded ER resident proteins accumulate in ER lumen.
- **Nucleophagy or Piecemeal Micro autophagy of Nucleus (PMN)**: Only a portion of nucleus is enveloped by autophagosomal membrane and finally gets degraded.
Mitochondrion

3-MA

Wortmannin

Type 1
(nutrient-deprivation)

PI3K
Beclin1

GFP-LC3

MPT

Type 2
(photo-damage)

Pink1
Parkin

MPT

Type 3
(micro-mitophagy)

Mitochondria-Derived Vesicles

<10 min

Mitophagosome

30-60 min

Mitophagosome

Pink1
Parkin

Multivesicular Body

Lysosome

Autolysosome
Molecular Mechanisms of Mitophagy
Mitophagy via autophagy adaptors or autophagy receptors
Mitochondrial ROS and MnSOD on cellular processes

Cartoon structure of MnSOD
Research Group and Support

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