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Research paper

Reduced tumor burden through increased oxidative stress in lung adenocarcinoma cells of PARP-1 and PARP-2 knockout mice

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ABSTRACT

Lung cancer (LC) is currently a major leading cause of cancer deaths worldwide. Poly(ADP-ribose) polymerases (PARP)-1 and −2 play important roles in DNA repair and other cell functions. Oxidative stress triggers autophagy and apoptosis. PARP inhibitors are currently used as anticancer strategies including LC. We hypothesized that inhibition of either PARP-1 or -2 expressions in the host animals influences tumor burden through several biological mechanisms, mainly redox imbalance (enhanced oxidative stress and/or decreased antioxidants, and cell regulators) in wild type (WT) lung adenocarcinoma cells. Compared to WT control tumors, in those of Parp-1$^{-/-}$ and Parp-2$^{-/-}$ mice: 1) tumor burden, as measured by weight, and cell proliferation rates were decreased, 2) oxidative stress levels were greater, whereas those of the major antioxidant enzymes were lower especially catalase, 3) tumor apoptosis and autophagy levels were significantly increased, and 4) miR-223 and nuclear factor of activated T-cells (NFAT)c-2 expression was decreased (the latter only in Parp-1$^{-/-}$ mice). Furthermore, whole body weight gain at the end of the study period also improved in Parp-1$^{-/-}$ and Parp-2$^{-/-}$ mice compared to WT animals. We conclude that PARP-1 and -2 genetic deletions in the host mice induced a significant reduction in tumor burden most likely through alterations in redox balance (downregulation of antioxidants, NFATc-2 and miR223, and increased oxidative stress), which in turn led to increased apoptosis and autophagy. Furthermore, tumor progression was also reduced probably as a result of cell cycle arrest induced by PARP-1 and -2 inhibition in the host mice. These results highlight the relevance of the host status in tumor biology, at least in this experimental model of lung adenocarcinoma in mice. Future research will shed light on the effects of selective pharmacological inhibitors of PARP-1 and PARP-1 in the host and tumor burden, which could eventually be applied in actual clinical settings.

1. Introduction

Lung cancer (LC) is the most prevalent cancer nowadays. Moreover, it is the leading cause of cancer deaths worldwide, with an overall survival rate lower than 15% in five years [1,2]. Cigarette smoke and other noxious particles that promote lung carcinogenesis are probably the main etiologic factors of LC [3,4]. Despite that the study of LC has received much attention in the last years, the lack of appropriate methodologies for the early diagnosis together with the absence of safe targeted therapies, are probably the main reasons accounting for its poor prognosis [5–10].

Poly(ADP-ribosyl)ation is a posttranscriptional modification by which poly(ADP-ribose) polymerases (PARPs) polymerize poly(ADP-ribose) on acceptor proteins using NAD^+ as a substrate [11]. PARP-1 and PARP-2 are the most relevant components of the base excision repair system, and repair single-stranded DNA breaks, thus maintaining genome stability [12]. Additionally, PARP-1 and PARP-2 also participate in other biological functions such as

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angiogenesis and transcription [12,13], apoptosis of oxidative-stress related pathologies [14–16], and regulation of the immune response [11,12]. On the other hand, an increased activity or over-expression of PARP-1 and -2 may also induce cellular damage by depleting the ATP stores of cells in several conditions [17–20] or by altering the mechanisms of DNA repair, leading to tumorogenesis of several cancer types [12,21–24]. Therefore, the role of PARP-1 and PARP-2 in cells is two-fold: while they play a critical role in DNA repair, they may also favor carcinogenesis and tumor progression [12,21–25]. In line with the latter, PARP-1 overexpression was also shown to correlate with poor survival of patients with breast cancer [26].

Pharmacological inhibitors of PARP-1 and -2 were also demonstrated to be novel therapeutic targets for the treatment of several cancer types including LC [12,21–24,27–31]. Furthermore, the association of PARP inhibitors with cisplatin also showed to have additive effects on the treatment of LC as well as cervical, liver, and testicular cancers [27,28,30–32]. The precise mechanisms whereby PARP-1 and -2 inhibitors may reduce tumor size and growth remain to be fully understood.

Oxidative stress, defined as the imbalance between oxidant production and antioxidant activity in favor of the former is involved in the pathophysiology of a wide range of acute and chronic conditions including LC [33–36]. For instance, several mechanisms led to increased oxidative stress in LC tumorogenesis, resulting in the accumulation of reactive oxygen species (ROS) such as hydroxyl radicals and superoxide anion [35,37]. Besides, oxidative stress also triggers several responses in cells that may trigger autophagy [38,39] and cell death [40]. Enhanced oxidative stress also induced DNA damage in tumor cells, which involved the activation of PARP-1 and PARP-2 molecules [15,41], and miR-223 targets PARP-1 expression in cancer [42] and other conditions [43]. Collectively, these mechanisms it could be used as a potential target mechanism in cancer therapy. Moreover, overactivation of PARP-1 through increased oxidative stress was also shown to promote apoptosis and necrosis in cells [44,45]. Recent evidence also indicates that PARP-1 activation favors autophagy through enhanced oxidative stress in in vitro models [46,47]. Unpublished observations from our group have recently shown that levels of several markers of oxidative stress were increased in the subcutaneous tumors of lung adenocarcinoma cells in mice. Additionally, the size of the tumors was significantly reduced and several markers of tumor growth were decreased in mice that were genetically deficient for either PARP-1 or -2 bearing the same lung adenocarcinoma [48].

On the basis of these observations, we hypothesized that inhibition of either PARP-1 or -2 expressions in the host animals may influence tumor burden through several biological mechanisms such as redox imbalance (increased oxidative stress and/or reduced antioxidants and several cell regulators), autophagy, and apoptosis in the wild type lung adenocarcinoma cells. We reasoned that as it happens in patients receiving anticancer therapies, the host is likely to play a relevant role in tumor growth and progression regardless of the cancer microenvironment and cell differentiation degree [49]. Accordingly, the main objectives in the current investigation were to assess in the lung adenocarcinoma tumors of Parp-1$^{-/-}$ and Parp-2$^{-/-}$ mice, the levels of oxidative stress, antioxidant enzymes, apoptosis, autophagy, and cell proliferation rates compared to tumors in wild type (WT) animals.

2. Methods (See additional information on all the methodologies in the online supplementary material)

2.1. Animal experiments

2.1.1. Tumor

The LP07 cell line derives from the P07 lung tumor, which spontaneously arose in the lungs of BALB/c mice [50]. Moreover, one month after tumor transplantation, all animals developed lung metastasis and spleen enlargement without affecting other organs [36].

2.1.2. Mice

BALB/c mice (2 months old, 20 g average weight) were obtained from Harlan *Interfauna Ibérica SL* (Barcelona, Spain). Parp-1$^{-/-}$ and Parp-2$^{-/-}$ mice (strain 129/Sv x C57BL/6), kindly provided by Dr. de Murcia [51] (Strasbourg, France), were backcrossed on BALB/c background for twelve generations. Genotyping was performed by PCR analysis of DNA from the tail vein as previously described [52]. All experiments were performed in 2 month's old (weight ~20 g) female mice, on a BALB/c background.

2.1.3. Experimental design and ethics

In all experimental groups, LP07 viable cells (4×10^5) resuspended in 0.2 mL minimal essential media (MEM) were subcutaneously inoculated in the left flank of female BALB/c mice on day 1 and were studied for a period of one month. This was a controlled study designed in accordance with the ethical regulations on animal experimentation (EU 2010/63 CEE, *Real Decreto* 53/2013 BOE 34, Spain) at *Parc de Recerca Biomèdica de Barcelona* (PRBB) and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986). All animal experiments were approved by the Animal Research Committee at PRBB (protocol number EBP-09-1228).

2.2. In vivo measurements in the mice

Body weight and food intake were measured every day during the 30 days of the study period. Moreover, food and water were supplied ad libitum for the entire duration of the study protocol.

2.3. Sacrifice and sample collection

On day 30 post-inoculation of LP07 tumor cells, animals from all experimental groups were sacrificed immediately after an intraperitoneal injection of 0.1 mL sodium pentobarbital (60 mg/kg). The subcutaneous tumors were obtained subsequently afterwards. In all mice, tumor weights were determined using a high-precision scale. Frozen tumors were used for immunoblotting techniques, while paraffin-embedded tumors were used for immunohistochemical experiments.

2.4. Tumor biology analyses

2.4.1. Immunoblotting of 1D electrophoresis

Protein levels of the different molecular markers and loading controls (Figures E1-E3) analyzed in the current investigation were evaluated according to methodologies published elsewhere [53].

2.4.2. Immunohistochemistry

In the tumor specimens from all groups, the cell proliferation marker ki-67 was identified on the 3-μm tumor paraffin-embedded sections using a specific antibody and immunohistochemical procedures as previously described in our group [36]. In addition, the number of positively stained nuclei for Ki-67 was counted in