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Review

Crosstalk between DNA repair and cancer stem cell (CSC) associated intracellular pathways

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ABSTRACT

DNA damaging agents (ionizing radiation and chemotherapeutics) are considered as most effective in cancer treatment. However, there is a subpopulation of carcinoma cells within the tumour demonstrating resistance to DNA damaging treatment approaches. It is suggested that limited tumour response to this kind of therapy can be associated with specific molecular properties of carcinoma stem cells (CSCs) representing the most refractory cell subpopulation. This review article presents novel data about molecular features of CSCs underlying DNA damage response and related intracellular signalling.

1. Introduction

Radiation therapy and various modern chemotherapeutics that are commonly used in cancer treatment, are DNA damaging agents. Targeting the DNA of carcinoma cells is suggested as one of the most effective anti-tumour approaches. While it is known that not all carcinoma cells could be killed by DNA damaging agents, it is also known that even not all cells are effectively inhibited in their proliferation by these agents. Mechanisms helping cells to escape cytostatic and cytotoxic effects after application of DNA damaging treatment approaches are not clearly understood and should be elucidated. Cancer stem cell (CSC) theory could explain how CSCs overcome cell death caused by DNA damaging agents. CSCs are defined as the most treatment resistant cell subpopulation with capacities for self-renewal and metastatic spread that maintains growth of primary and secondary tumours [1]. It is also suggested that CSCs possess specific intracellular molecular properties helping to avoid treatment-caused cytotoxicity. Understanding the reasons of CSC resistance to DNA damaging agents can help to predict and improve therapy response and clinical outcome in cancer patients treated with radiotherapy and/or DNA-targeting chemotherapeutics.

2. Treatment-induced DNA damage in CSCs

As mentioned above, CSCs is a persistent intratumoral cell subpopulation responsible for tumour formation, therapy resistance, metastatic spread and local and distant recurrence development. Despite an opinion that DNA damaging agents (ionizing radiation, chemotherapeutics) are the most effective anti-cancer approaches, CSCs could successfully survive and initiate tumour re-growth. CSC survival may be observed due to mobilization of DNA repair mechanisms after administration of DNA damaging treatment approaches. To protect cellular DNA, following DNA repair pathways are activated in cancer cells: double-strand breaks (DSBs), base excision repair (BER), transcription-coupled nucleotide excision repair (NER), and mismatch repair (MMR).

It is generally believed that CSCs are characterized by significant enhancement of DNA repair mechanisms. Understanding the mechanisms of the DNA repair in CSCs could provide new opportunities to sensitize CSCs to DNA damaging agents. This review article will consider BER and DSBs repair mechanisms in the context of CSC-specific intracellular molecular perturbations.

3. Base excision repair (BER) and CSC molecular properties

BER is one of the major DNA repair pathways involved in the removal of the base damages caused by ionizing radiation, oxidative or alkylating agents, and also endogenous or exogenous daily adducts [2,3]. Incorrect or non-repaired damaged bases of DNA are removed by DNA glycosylase that could be considered as the initial step of the BER pathway. The next enzyme that is activated in the BER pathway is apurinic/apirimidinic endonuclease/redox

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effector factor (Ape1/Ref-1, also known as APEX1) [4]. Ape1/Ref-1 is a ~37 kDa protein containing two distinct domains. Thus, the N-terminal domain is essential for redox activity and the C-terminal domain for endonuclease activity. It is known that both domains are important and required for DNA repair.

During cell exposure to DNA damaging agents (ionizing radiation, bleomycin, platinum compounds, etc.) expression of Ape1/Ref-1 is increased due to the treatment-caused production of reactive oxygen radicals (ROS) and stimulation of AP endonuclease activity [5]. Additionally, it was recently demonstrated that carcinoma cells with primary or secondary resistance to the DNA damaging agents (irradiation, platinum agents) also reveal overexpression of Ape1/Ref-1 [6–8]. Since Ape1/Ref-1 is implicated in the development of treatment resistance in carcinoma cells through its dual functions, such as enhancement of DNA repair and ROS scavenging, it is logical to suggest that Ape1/Ref-1 is also involved in CSC activities. CSCs have unique abilities to maintain low intracellular ROS levels in order to protect themselves from ROS-caused DNA damage, senescence or cell death [9–11]. Low ROS levels in CSCs are maintained due to elimination of ROS by activated intracellular scavenging systems. Ape1/Ref-1 is an effective ROS scavenger and its expression depends on the constitutive or treatment-caused intracellular levels of ROS. Constitutive up-regulation of Ape1/Ref-1 may be observed due to permanent ROS production caused by microenvironmental factors in the CSC niche (hypoxia, release of inflammatory cytokines, etc.). This constitutive Ape1/Ref-1 overexpression effectively protects CSCs from ROS appearing after administration of DNA damaging agents. Hence, Ape1/Ref-1 could be suggested as a reason for the primary treatment resistance of CSCs due to the immediate elimination of ROS, and to CSC protection from ROS-associated cell damage. Acquired therapy resistance to DNA damaging agents is accompanied by additional activation and up-regulation of already overexpressed Ape1/Ref-1 in CSCs in response to treatment-caused ROS formation [6].

It was additionally shown that owing to redox function, Ape1/Ref-1 could also be implicated in the CSC differentiation [9,12]. Alteration of ROS levels and redox homeostasis can result in the enhancement of either CSC self-renewal or differentiation. Thus, low levels of ROS are critical for CSC self-renewal, whereas higher levels of ROS markedly inhibit self-renewal and lead to CSC differentiation [9]. Therefore, Ape1/Ref-1 could be considered as one of the key regulators of CSC aggressiveness with altered differentiation signalling. Gurusamy et al. reported that inhibition of redox function of Ape1/Ref-1 combined with H_2O_2 treatment led to the enhancement of cardiac stem cell differentiation, followed by apoptosis development [13]. It is generally believed that concomitant use of DNA damaging agents and specific inhibitors of the Ape1/Ref-1 redox domain could potentially increase intracellular ROS levels associated with enhanced CSC differentiation, followed by cell death (Fig. 1).

Ape1/Ref-1 is also reported as a transcriptional regulator modulating the redox status of a variety of transcriptional factors, including p53 protein [9,14]. Tell et al. noted that proteome-based experiments with silenced Ape1/Ref-1 showed activation of p53 with concomitant perturbations of the receptor-related intracellular signalling [14,15]. It was additionally demonstrated that p53 regulated ROS and Ape1/Ref-1 play critical roles in CSC self-renewal and differentiation, and in CSC death and survival [15,16]. Owing to the Ape1/Ref-1-caused reduction of oxidized p53, enhancement of p53's DNA-binding function could be observed in carcinoma cells [8]. Therefore, redox modulation of p53 significantly contributes to DNA repair in CSCs.

Intratumoral hypoxia also plays an important role in the generation of carcinoma cells with CSC properties through ROS formation, regulation of DNA-binding activity of HIF-1α and enhancement

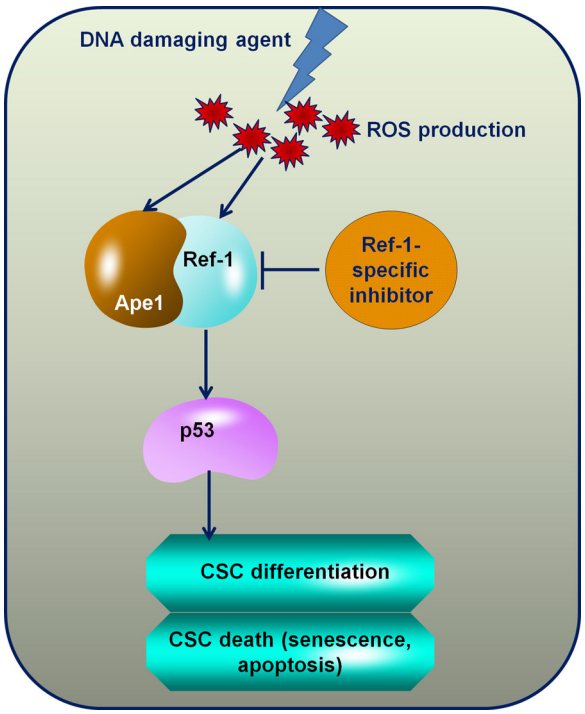

Fig. 1. Specific inhibition of the Ref-1 domain enhances the efficacy of DNA damaging agents. Administration of DNA increases ROS production in carcinoma cells accompanied by amplification of ROS scavenger Ape1/Ref-1 and activation of the Ref-1 redox domain. Overexpressed and activated Ref-1 domain decreases ROS levels in carcinoma cells, resulting in the enhancement of carcinoma cell stemness and self-renewal. In contrast, specific inhibition of the Ref-1 domain responsible for ROS scavenging is accompanied by increased intracellular ROS levels, activation of p53, repression of cell self-renewal and promotion of cell differentiation followed by cell death. Hence, Ref-1 blockers can be used in combination with DNA damaging agents to improve their anti-tumour effects.

of genome instability [8,17–19]. Since Ape1/Ref-1 is required for redox regulation of HIF-1α, this molecule could be considered as an upstream regulator of DNA repair genes controlled by HIF-1α [8].

Furthermore, Ape1/Ref-1 is involved in the regulation of Rac GTPase activity in carcinoma cells [18,20]. Rac1 has been recently suggested as a protein that is closely associated with CSC formation and activity, and with carcinoma cell resistance to ionizing radiation and cisplatin [21–24]. Rac1 is also responsible for carcinoma cell motility and migration [25–27], so it is possible to assume that Rac1 regulation via Ape1/Ref-1 could modulate the metastatic potential of CSCs, as well as their sensitivity to radiation therapy and DNA damaging chemotherapeutics (cisplatin). Rac1 was shown to be implicated in the BER processes [28–30]. Thus, accumulation of ROS-induced DNA base lesions was accompanied by increased activity of Rac1. It was already shown that enhancement of Rac1 activity was accompanied by Rac1 translocation into nuclei, and by activation of G2-M checkpoints resulting in prolongation of the G2-M phase of the cell cycle [24,31,32]. These intracellular events allow carcinoma cells to repair damaged DNA. Unfortunately, this kind of DNA repair additionally increases DNA instability and the number of DNA mutations, thus contributing to processes of carcinogenesis and CSC formation. Furthermore, activation of Rac1 results in activation via phosphorylation of the Ras downstream targets Raf1, MEK1/2 and ERK1/2 that promote enhancement of carcinoma cell aggressiveness, insensitivity to therapeutic approaches and development of metastatic disease [28].

Taken together, the intracellular events described here might be very important regulators of CSC fate. Hence, these signallings should be further investigated to identify the most potential and