MINI-REVIEW

Radiation-induced Cochlea Hair Cell Death: Mechanisms and Protection

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Abstract

Cochlea hair cell death is regarded to be responsible for the radiation-induced sensorineural hearing loss (SNHL), which is one of the principal complications of radiotherapy (RT) for head and neck cancers. In this mini-review, we focus on the current progresses trying to unravel mechanisms of radiation-induced hair cell death and find out possible protection. P53, reactive oxygen species (ROS) and c-Jun N-terminal kinase (JNK) pathways have been proposed as pivotal in the processes leading to radiation hair cell death. Potential protectants, such as amifostine, N-acetylcysteine (NAC) and epicatechin (EC) , are claimed to be effective at reducing radiation-inducedhair cell death. The RT dosage, selection and application of concurrent chemotherapy should be pre-examined in order to minimize the damage to cochlea hair cells.

Keywords: Radiotherapy - sensorineural hearing loss - ototoxicity - hair cell - apoptosis

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Introduction

Radiotherapy (RT) is one of the important treatments for head and neck cancers, especially for nasopharyngeal carcinoma (NPC), while the auditory pathway is often involved in the radiation fields. Radiation-induced sensorineural hearing loss (SNHL), which is permanent and badly affecting patients' quality of life, has long been recognized as a principal complication of RT for head and neck cancers. In patients who have received radiotherapy for NPC, the incidence of eventual SNHL is reportedly as high as 50% (Low and Fong, 1998). Several studies have proved that the death of cochlea hair cell is responsible for the radiation-induced SNHL (Bohne et al., 1985; Honore et al., 2002; Linskey and Johnstone, 2003; Low et al., 2005; Li et al., 2010). In order to prevent SNHL, it is very important to thoroughly understand the mechanisms and protections of radiation-induced cochlea hair cell death.

Mechanisms of radiation-induced cochlea hair cell death

Radiation can have multiple effects on cells by disrupting chemical bonds in all the basic components of cells such as lipids, proteins and most importantly, in the genetic compartment of the cell, the DNA, which is the key point in radiation-induced cell death (Iliakis, 1991). Cell death in general is an extremely heterogeneous process. It is common for the same cell population occurring multiple cell death pathways at the same time (Jiang et al., 2006), including apoptosis, autophagy, necrosis and mitotic catastrophe (Selzer and Hebar, 2012). So far, nearly all of the researches on radiation-induced hair cells death focus on apoptosis that is generally accepted as an important mechanism of radiation-induced cell death in vivo (Verheij and Bartelink, 2000). It has been shown by Wong-Kein Low et al. (2006b) that apoptosis occurred mainly at 72 h post-irradiation for both 5 and 20 Gy and the extent of apoptosis was dose-dependent - the higher the dose of radiation applied, the greater the extent of apoptosis occurred. The pathways leading to apoptosis in hair cells has been partially elucidated. Some key regulators associated with apoptosis such as p53, reactive oxygen species (ROS) and c-Jun N-terminal kinases (JNK), are recently shown to play important roles in radiated hair cell death.

P53

When the radiation-induced DNA damage overwhelms the cell's intrinsic DNA repair mechanisms, apoptosis mediated by p53 is initiated. P53, a well-known cellular regulator, has been shown to be an upstream regulator of cisplatin-induced hair cell death (Cheng et al., 1999). Deletion of the p53 gene protects sensory hair cells from cisplatin-induced cell death, caspase-3 activation, and cytochrome c translocation. After p53 phosphorylation, the apoptotic-related genes including Bcl-2 family member, Bax (Ferri and Kroemer, 2001), are transcriptionally up-regulated. It is known that Bcl-2 family members can be characterized as either anti-apoptotic or pro-apoptotic. One of the important pro-apoptotic members is Bax, which can translocate from the cytoplasm to the mitochondria

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and promote the formation of pores in the mitochondrial membrane leading to the generation of ROS, and leakage of cytochrome c into the cytoplasm (Cheng et al., 2005). Activated nuclear p53 can also translocate directly to and damage the mitochondria, where it can physically interact with and inactivate prosurvival Bcl-2 proteins (Marchenko et al., 2000). The up-regulation of p53 is observed in hair cells at 72 h after 5 and 20 Gy of radiation. Phosphorylation of p53 protein was increased dramatically at 3 h post-irradiation for both 5 and 20 Gy. Several p53-regulated genes associated with cell cycle regulation and arrest were also found increased corresponding to the phosphorylation of p53 (Low et al., 2006b). Although the relevance between p53 and hair cell degeneration has been proposed, the exact role of p53 and its downstream in regulating radiation-induced hair cell death remain elusive to our knowledge.

ROS

Although the relationship between ROS and apoptosis has not yet been clearly brought to light, ROS is believed to play a key role in the promotion of apoptosis by affecting mitochondrial permeability, release of cytochrome c and activation of p53, caspases, JNK and p38 mitogen activated protein kinases (MAPK) pathways in auditory cells (Devarajan et al., 2002; Tabuchi et al., 2011). A recent study demonstrated low concentrations of H_2O_2 mortally injured the hair cells by reducing mitochondrial membrane potential and mitochondrial produced antioxidants (Baker and Staecker, 2012). There are increasing evidences implicating ROS in the damage associated with cochlear ischemia, noise trauma, aminoglycoside and CDDP ototoxicity (Seidman and Vivek, 2004). It has also been shown that the generation of intra-cellular ROS correlates with the dose of radiation applied. Exposing hair cell line OC-k3 to different doses (2, 20, 100 Gy) of radiation resulted in a dose-dependent increase in the fluorescence of the oxidation-sensitive probe determined at 1 h post-irradiation (Low et al., 2006b). Furthermore, ROS was postulated to cause preferential damage of high frequency hearing by radiation (Fleury and Lapeyre, 2010). It has been shown that the basal (respond to higher frequency sounds) outer hair cells may be more susceptible to free-radical damage than the apical (respond to lower frequency sounds) outer hair cells due to a significantly lower level of the antioxidant - glutathione in the basal outer hair cells compared with the apical outer hair cells (Sha et al., 2001). Evidences show that accumulation of more ROS often results more severe damage of the hair cells.

JNK

The JNKs are also called stress activated protein kinases (SAPKs) and are members of the family of MAPK. The JNKs are activated in response to a variety of cellular insults and play important roles in development, apoptosis, cell growth, inflammatory, and immune responses (Sabapathy, 2012). When activated, JNKs phosphorylate and activate the transcription factor, c-Jun, which leads to either promotion or prevention of apoptosis. Several studies specifically identified the JNK signaling pathway as a mediator of apoptosis in outer hair cells (Wang et al., 2007). The activation of c-Jun is regarded as a pivotal event in the JNK-mediated apoptosis of oxidative stress-damaged cochlea hair cells following exposure to either acoustic trauma or a toxic level of an aminoglycoside antibiotic (Zine and van de Water, 2004). This leads us to think that JNK may also play an important role in irradiated hair cells. J. H. Pyun et al. (Pyun et al., 2011) found that the expression of p-JNK was significantly up-regulated in hair cell line HEI-OC1 within 24h post-radiation, which led to the activation of c-Jun. Another research directed by Wong-Kein Low (Low et al., 2006b) analyzed the expression of c-Jun but not the JNK in hair cell line OC-k3 after irradiation. Microarray analysis demonstrated down-regulation of the c-Jun gene at 24h post-radiation, but not at 72 h or longer. However western blotting revealed that the activations of c-Jun and phospho-c-Jun took place earlier at 3h post-radiation, but not at 48h or longer. Although c-Jun activation occurred as early as 3 h post-irradiation, it was not sustained with time. These results suggested that the c-Jun pathway may not lead to radiation-induced apoptosis. Wong-Kein Low then proposed that the JNK pathway may play a protective role in radiation-induced cochlear cell damage similar to its role in the CDDP-induced hair cell death. Instead of inducing apoptosis in the latter, JNK promotes DNA repair and maintenance of CDDP-damaged sensory cells (Wang et al., 2004), possibly by down-regulating the expression of PTEN (Hettinger et al., 2007). Currently it is still under debate that the exact role of JNK plays in apoptosis of hair cells. Due to the complexity of multiple JNK forms involving in multiple cellular processes in various cell types (Sabapathy, 2012), more studies should be done to elucidate the exact relationship between JNK and post-radiation hair cell death.

Caspases

Caspases is a family of proteases that exist normally in an inactive form and can be activated by cleavage of an inactivating prodomain. The productions of these mature enzymes lead to cellular apoptosis. Caspase-9 (as an upstream caspase) and caspase-3 (as a downstream caspase) have been shown to be key mediators of cochlea hair cell death induced by drugs and noise (Cheng et al., 2005). The interactions between them and other species such as ROS, p53 and cytochrome c from the mitochondria in hair cells have been reported in several studies (Devarajan et al., 2002; Cheng et al., 2005; Tabuchi et al., 2011). But studies on their roles in radiated hair cells are very limited. Assuming the apoptosis of hair cells may share a common pathway, caspase-3 and -9 could play important roles in radiation induced hair cells death.

Protections of radiation-induced cochlea hair cell death

Normally when the depletion of functioning cells reaches a critical level, a clinically detectable effect becomes apparent (Awwad, 1990). Because of the finite, post-mitotic and non-regenerating characteristics of the hair cells in the cochlea, radiation-induced SNHL may present when the dead hair cells reach a certain of number.

Understanding the importance to prevent hearing loss by maintaining the number of hair cells, researchers have done lots of studies trying to discover effective protectants and better treatment plans to minimize the injury to hair cells.

Potential protectants

Amifostine is probably the most widely studied and used radioprotective agent up-to-date (Bourhis et al., 2011). As a synthetic prodrug aminothiol, it reduces radiation-induced ROS through its free sulfhydryl moiety. After several important clinical trials, amifostine was approved by the US Food and Drug Administration and European Medicines Agency. It is recommended for routine use during the treatment to reduce xerostomia in patients with head and neck cancer undergoing RT (Hensley et al., 2009). It also has been widely used in preventing radiation induced mucositis and esophagitis, although the protective effect is still controversial. Given its selective cytoprotective effect and low toxicity against the harmful effects of radiotherapy, amifostine was studied by Ricardo (Lessa et al., 2009) to verify its protective effect in cochlea of irradiated guinea pigs. They found the extent of injury induced by irradiation was lower in the outer hair cells of the groups treated with amifostine compared to the group without treatment. Another group that received only amifostine without irradiation showed no hair cell damage at all. All these results suggest amifostine is safe and effective on protecting cochlea outer hair cells from radiation-induced cell death. The protective effect study of amifostine for radiated hair cells should be included in its ongoing clinical trials.

N-Acetylcysteine (NAC) or its metabolically deacetylatable form L-NAC, is a naturally occurring compound found in several vegetables including garlic, onion, peppers and asparagus (Mansour et al., 2008). As a thiol reducing agent, it has been proved capable of inhibiting chemically induced oxidative stress and DNA damage (Reliene et al., 2004). Since mid-1950s it has been used in clinical practice and proved safe even when used in relatively large doses (De Flora et al., 2001). The protective effect of L-NAC on the cochlea by suppressing the ROS pathway has been studied in CDDP (Bertolaso et al., 2001) and radiation (Low et al., 2008) induced SNHL. Wong-kein Low et al. (2008) demonstrated that L-NAC protected against radiation induced apoptosis by inhibiting intracellular ROS in cochlea hair cell line OC-K3. In an animal experiment, NAC was found capable of maintaining the amount and function of cilium in hair cells of guinea pigs after 70 Gy cranium radiation (Xie et al., 2012). Study on the direct protective effect of NAC on radiated tissues, especially on cochlea hair cells would be very helpful. Furthermore L-NAC was demonstrated to be suitable for delivering directly to the inner because it's small enough to pass through the round window membrane (Feghali et al., 2001). Thus it may be feasible to administrate NAC locally in the inner ear through Eustachian tube, which may minimize the systematic side effects and interferences to RT.

Epicatechin (EC), extracted from green tea, was reported to prevent cisplatin-induced ototoxicity caused by ROS generation as well as changes in the mitochondrial membrane potential (MMP) (Kim et al., 2008). J. H. Pyun et al. (2011) also demonstrated the inhibition of radiation-induced apoptosis in cochlea hair cells by EC. After treating with EC combined with radiation, significant increase of cell viability, decrease of apoptosis, inhibition of ROS generation and reduction of signals related to apoptosis were observed in hair cell lines HEI-OC1 and UB-OC1. In addition, the safety of EC has been proved by its attenuating the radiation-induced embryotoxicity and protecting against radiation-induced loss and changes of auditory neuromast in the zebrafish, as well as, inhibiting the hearing threshold shift against hearing loss in radiated rat models. All of these evidences suggest EC may be a safe and effective agent for the prevention of radiation-induced SNHL.

Superoxide dismutase (SOD) is considered as one of the most important antioxidant enzymes. It effectively catalyzes the conversion of $\bullet O^{2-}$ to H_2O_2 and thereby controls the concentration of superoxide (Mikkelsen and Wardman, 2003). EUK-207, a SOD/Catalase mimetic, is found to mitigate radiation dermatitis and promote wound healing in irradiated rat skin (Doctrow et al., 2012). In addition it is also found to mitigate pneumonitis and fibrosis after irradiation (Gao et al., 2012) in animal experiments. It is speculated that EUK-207 may also protect cochlea from radiation injury just as it does in radiated skin and lung.

A promising radioprotection pathway, stat3/socs3a pathway, was investigated recently by J. Liang et al. (2012). They demonstrated that the stat3/socs3a pathway is a key regulator of hair cell regeneration in zebrafish. In that research properly activation of stat3/socs3a has been shown to promote hair cell regeneration through stem cell activation, cell division, and differentiation. It is reasonable to speculate stat3 may be at least one of the important repairing factors in hair cells after irradiation. Thus stat3 activation could be a potential therapeutic to radiation induced hair cell death.

Radiation dose limitation

As the effective hair cells protectants are still under studied, reducing or avoiding the radiation to cochlea may be a main strategy for preventing cochlea hair cells death at present. Total dose to the cochlea seems to be a significant factor though fractionation, age and chemoradiation may also contribute to ototoxicities (Bhandare et al., 2007). Thus limiting the dose delivered to the cochlea seems critical. For conventionally fractionated RT or IMRT to minimize the risk for SNHL, the mean dose to the cochlea was suggested to be limited to 45 Gy (Pan et al., 2005; Chen et al., 2006), or more conservatively 40 Gy (Fleury and Lapeyre, 2010). In case of association with other causes of toxicity (such as age, low baseline value, association to cisplatin), this dose should be as low as possible (Fleury and Lapeyre, 2010). For children, a cumulative cochlear dose less than 35 Gy is recommended for patients planned to receive 54-59.4 Gy in 30-33 treatment fractions (Hua et al., 2008).

Concurrent chemotherapy selection

In the treatment of some head and neck cancers, such as nasopharyngeal carcinoma, concurrent chemotherapy is strongly recommended, which strengthens the curative effect of radiation but also intensifies the radiation side-effect remarkably at the same time. Patients with NPC who received RT and concurrent/adjuvant chemotherapy using cisplatin (CDDP) are found experiencing greater SNHL compared with patients treated with RT alone, especially to high-frequency sounds in the speech range (Low et al., 2006a). Cell viability was greatly more reduced in combined CDDP-RT on cochlea hair cell lines than CDDP or RT alone (Low et al., 2010). Given both chemotherapy and RT have hair cell toxicity, radiation dose constraints and chemotherapy dose intensity should be considered. High-frequency SNHL was found profoundly damaged in head and neck cancers patients who received concomitant cisplatin with doses of 100 mg/m2/3 weeks compared to 40 mg/m2/1 weekly (Hitchcock et al., 2009). The threshold cochlear dose for hearing loss with CDDP-based chemotherapy and RT was estimated to be 10 Gy (Hitchcock et al., 2009), which is far from the curative dose however. In the chemo-radiotherapy treatment for NPC, the incidence of high-frequency SNHL is significant higher in patients treated with CDDP than those with carboplatin (Petsuksiri et al., 2011). It seems carboplatin may be more recommendatory if its curative effect is equal to CDDP, which is currently the most commonly used in head and neck cancers. After all, in order to minimize the radiation-induced hair cell death, the radiation dose to the cochlea should be as low as possible and carboplatin is recommended over CDDP in case concurrent chemotherapy is required.

Concluding remarks

Although a great deal of researches have been done to elucidate the mechanism of cochlea hair cell death induced by drugs or noise, the researches on radiation induced hair cell death is very limited. As mentioned above, the p53, ROS and JNK mediated apoptosis of hair cells may be pivotal in the process to SNHL in spite of lacking hard evidences. Further studies are still needed to figure out the exact roles and interactions of these pathways. Other well-known apoptosis associated mediators, such as caspase-3, caspase-9 and Bax, which are fully investigated in other tissues may also play important roles in hair cell death.

Most of the radiated hair cells protectants are functioning as inhibitors of ROS generation and proved to be effective. Although antioxidants are established as protectants, their connection with other cell death events remains elusive. And no matter how efficacious the protectant is, a prerequisite for the successful use of an anti-apoptotic treatment is that it must be safe and must not interfere with the efficacy of RT to cancer. Because these findings are based on immortalized cochlear cell lines or experimental animals, the practical safe ranges of dosage for human beings need to be further determined. Given the fact that oxidation can improve sensitivity of tumor to RT, it's possible that the inhibition of ROS in hair cells may also affect the efficacy of RT to tumor bed. The use of target-specific approach may dispel that misgiving. Access of protectants to the inner ear could possibly be achieved through diffusion from the middle ear where the drugs could be applied through the tympanic membrane via the external ear canal (Seidman and Vivek, 2004) or through Eustachian tube if the molecule is small enough. Nevertheless, we are still far away from discovering a protectant for radiated hair cells that could be eventually translated into clinical routine uses.

As the technology of RT improved, three-dimensional conformal radiotherapy (3D-CRT) and intensity-modulated radiotherapy (IMRT) have been widely used, which are allowed for better dose conformation, improving dose escalation to the target and sparing of more normal tissues comparing with conventional radiotherapy. Although the incidence of radiation-induced hair cell death may reduce to a certain extent, it cannot be avoided completely. Since RT and concurrent chemotherapy will exacerbate the impairment of hair cells, the limitation of RT dose, selection and application of chemotherapy should be well defined beforehand. In the future the invention of tumor targeting drugs, which may kill the cancer cells precisely but avoid the normal cells, may allow a less RT dosage resulting in less toxicity to hair cells. All in all, in combination of the advanced technologies, the better understanding of the mechanisms of radiation-induced cochlea hair cell death, the better protections we will have.

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