Introduction

Hearing level is affected by genetic, aging and environmental factors [1, 2]. This review focuses on genetic factor-mediated hearing loss. About 120 million people worldwide suffer from congenital (early-onset) hearing loss. Thirty percent of them have syndromic hearing loss and the remaining 70% have non-syndromic hearing loss. In addition, a large number of elderly people worldwide suffer from age-related (late-onset) hearing loss. c-Ret and c-RET have been shown to be essential for the development and maintenance of neurons including the enteric nervous system (ENS) in mice and humans. Impairments of endothelin receptor B (EDNRB) and SOX10 have been shown to cause a significantly increased risk of dominant sensorineural deafness in Hirschsprung disease (HSCR) patients. We have recently shown that impairments of tyrosine 1062 (Y1062) phosphorylation in c-Ret causes syndromic congenital deafness in mice and humans and non-syndromic age-related hearing loss with neurodegeneration of spiral ganglion neurons (SGNs) in mice. This review focuses on the pathogenesis of hearing loss caused by impairments of c-Ret.

Keywords: c-Ret, congenital deafness, age-related deafness, tyrosine kinase, spiral ganglion neuron, neurodegeneration

Review Article
c-Ret-mediated hearing losses

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Abstract: About 120 million people worldwide suffer from congenital (early-onset) hearing loss. Thirty percent of them have syndromic hearing loss and the remaining 70% have non-syndromic hearing loss. In addition, a large number of elderly people worldwide suffer from age-related (late-onset) hearing loss. c-Ret and c-RET have been shown to be essential for the development and maintenance of neurons including the enteric nervous system (ENS) in mice and humans. Impairments of endothelin receptor B (EDNRB) and SOX10 have been shown to cause a significantly increased risk of dominant sensorineural deafness in Hirschsprung disease (HSCR) patients. We have recently shown that impairments of tyrosine 1062 (Y1062) phosphorylation in c-Ret causes syndromic congenital deafness in mice and humans and non-syndromic age-related hearing loss with neurodegeneration of spiral ganglion neurons (SGNs) in mice. This review focuses on the pathogenesis of hearing loss caused by impairments of c-Ret.

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c-Ret and hirschsprung disease (HSCR)

The c-RET proto-oncogene encodes a receptor-tyrosine kinase, and glial cell line-derived neurotrophic factor (GDNF) is one of the ligands for c-RET [6]. GDNF exhibits its effect on target cells by binding to a glycosyl phosphatidylinositol (GPI)-anchored cell surface protein (GFRα1). The binding leads to the formation of a complex with the receptor tyrosine kinase c-RET. Formation of this complex stimulates c-RET autophos-
phorylation as a trigger for c-RET-mediated signaling pathways to give positive effects for cell survival [6-9]. Previous studies have also indicated that there is a Ret-independent signaling pathway stimulated by GDNF [7, 10, 11]. Tyrosine 1062 (Y1062) in c-Ret is one of the most important autophosphorylation sites for its kinase activation and is a multi-docking site for several signaling molecules including SHC, a transmitter for c-Ret-mediated signaling pathways [10, 12, 13]. c-RET is responsible genes for HSCR as described above, including survival of neuronal cells [10, 30]. Our recent studies showed that c-Ret-KI Y1062F/Y1062F-mice had severe congenital deafness with neurodegeneration of SGNs that resulted in decreased numbers of SGNs on postnatal day (P) 8-18, while c-Ret-KI Y1062F/Y1062F-mice showed a comparable number and comparable morphology of SGNs to those in WT mice on P2-3 [1] (summarized in Figure 1). The level of Y1062 phosphorylation in c-Ret of SGNs from WT mice on P2-3 was undetectable, while that on P8-18 sharply rose [1]. These results suggest that SGNs even from c-Ret-KI Y1062F/Y1062F-mice developed normally at least until P3 after birth, when Y1062 phosphorylation in c-Ret of SGNs from WT mice was undetectable, but those from c-Ret-KI Y1062F/Y1062F-mice no longer survived on P8-18, when the level of Y1062 phosphorylation in c-Ret of SGNs from WT mice was high (summarized in Figure 1). On the other hand, partially impaired phosphorylation of Y1062 in c-Ret in SGNs facilitated age-related hearing loss with accelerated reduction of SGNs from 4 months of age, whereas it had almost no affect on hearing and cell density of SGNs at least until 1 month of age, when the auditory system had been maturated (summarized in Figure 1). The neurodegeneration of SGNs from c-Ret-KI Y1062F/Y1062F-mice did not involve the hallmark of apoptotic signals [1]. Our results partially correspond to results of previous studies showing that c-Ret, GFRα1 and GDNF are expressed in auditory neurons [19, 20] and that GDNF has a protective effect on antibiotics-induced ototoxicities [21-24]. In previous studies, mutations of cadherin 23 have been shown to correlate with early-onset syndromic hearing loss [25, 26] as well as non-syndromic hearing loss with different onsets [27-29] in mice and humans. Thus, if our data integrate the conclusions of those studies, different mutations in an identical gene can develop both early-onset syndromic and late-onset non-syndromic hearing losses. Our recent studies therefore provide a new concept for hearing research that early-onset syndromic hearing loss and late-onset non-syndromic hearing loss partially share a common pathogenesis that is monogenetically affected by a single point mutation (Y1062F) in c-Ret.

**c-Ret-mediated neurodegeneration of SGNs**

Y1062 phosphorylation in c-Ret has been reported to mediate several biological responses, including survival of neuronal cells [10, 30]. Our recent studies showed that c-Ret-KI Y1062F/Y1062F-mice had severe congenital deafness with neurodegeneration of SGNs that resulted in decreased numbers of SGNs on postnatal day (P) 8-18, while c-Ret-KI Y1062F/Y1062F-mice showed a comparable number and comparable morphology of SGNs to those in WT mice on P2-3 [1] (summarized in Figure 1). The level of Y1062 phosphorylation in c-Ret of SGNs from WT mice on P2-3 was undetectable, while that on P8-18 sharply rose [1]. These results suggest that SGNs even from c-Ret-KI Y1062F/Y1062F-mice developed normally at least until P3 after birth, when Y1062 phosphorylation in c-Ret of SGNs from WT mice was undetectable, but those from c-Ret-KI Y1062F/Y1062F-mice no longer survived on P8-18, when the level of Y1062 phosphorylation in c-Ret of SGNs from WT mice was high (summarized in Figure 1). On the other hand, partially impaired phosphorylation of Y1062 in c-Ret in SGNs facilitated age-related hearing loss with accelerated reduction of SGNs from 4 months of age, whereas it had almost no affect on hearing and cell density of SGNs at least until 1 month of age, when the auditory system had been maturated (summarized in Figure 1). The neurodegeneration of SGNs from c-Ret-KI Y1062F/Y1062F-mice did not involve the hallmark of apoptotic signals [1]. Our results partially correspond to results of a previous study.

**c-Ret-mediated syndromic and non-syndromic hearing losses**

Despite the fact that c-Ret and c-RET are responsible genes for HSCR as described above, there had been no direct evidence to link c-Ret and c-RET with deafness in mice and humans. Our recent results have shown that complete impairment of phosphorylated Y1062 in c-Ret without change in the expression level resulted in the development of congenital hearing loss in c-Ret-KI Y1062F/Y1062F-mice [1], while partial impairment of phosphorylated c-Ret resulted in normal hearing development until 1 month of age but afterwards acceleration of age-related hearing loss in c-Ret-KI Y1062F/Y1062F-mice [18] (summarized in Figure 1). Thus, impairments of c-Ret phosphorylation monogenetically cause not only early-onset syndromic hearing loss but also late-onset non-syndromic hearing loss. Our results partially correspond to results of previous studies showing that c-Ret, GFRα1 and GDNF are expressed in auditory neurons [19, 20] and that GDNF has a protective effect on antibiotics-induced ototoxicities [21-24]. In previous studies, mutations of cadherin 23 have been shown to correlate with early-onset syndromic hearing loss [25, 26] as well as non-syndromic hearing loss with different onsets [27-29] in mice and humans. Thus, if our data integrate the conclusions of those studies, different mutations in an identical gene can develop both early-onset syndromic and late-onset non-syndromic hearing losses. Our recent studies therefore provide a new concept for hearing research that early-onset syndromic hearing loss and late-onset non-syndromic hearing loss partially share a common pathogenesis that is monogenetically affected by a single point mutation (Y1062F) in c-Ret.

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c-Ret and hearing losses

showing neurodegeneration of spiral ganglion neurons in *endothelin receptor B* (*Ednrb*) homozygously deleted mice [31] and postmigratory enteric neurons without apoptotic signals during the developmental stage in mice with conditional ablation of c-Ret [32].

**c-Ret-mediated signaling pathway in spiral ganglion neurons**

Results of a previous *in vitro* study showed that phosphorylated levels of Y1062 in c-RET modulate Akt and NF-κB activities in neural cells [30].

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**Figure 1.** Schematic summary of c-Ret-mediated SGNs loss. (A) Inner ear from a wild-type (WT) mouse at postnatal day (P) 14 stained with hematoxylin and eosin. Red triangle, yellow square and blue square contain spiral ganglion neurons (SGNs), inner- and outer-hair cells and the stria vascularis, respectively. Our morphological analyses showed almost no abnormalities in these areas besides SGNs from c-Ret-KI Y1062F/Y1062F-mice. (B-E) Colored triangles in the schema represent Rosenthal’s canals in WT (yellow triangles), homozygous c-Ret-KI Y1062F/Y1062F (Ret KI (YF/YF), pink triangles) [1] and heterozygous c-Ret-KI Y1062F/+ -mice (Ret KI (YF/+), light blue triangles) [18]. Blue circles with a “black” line in the triangles represent SGNs. Blue circles with a “red” line in the triangles represent SGNs with “phosphorylated Y1062 in c-Ret”. X-axis and Y-axis indicate age of mice and hearing levels, respectively. (B) WT mice showed that c-Ret protein was constantly expressed in SGNs (P1-18), while the numbers of Y1062-phosphorylated SGNs (blue circles with “red” line in yellow triangles in B) were sharply increased around P6-7, several days before the WT mice begin to acquire intact hearing levels (around P12~) [1]. (C) c-Ret-KI Y1062F/Y1062F-mice suffered from congenital deafness with decreased cell density of SGNs. (C) c-Ret-KI Y1062F/Y1062F-mice showed no Y1062-phosphorylated SGNs even on P8, although Y1062-phosphorylated SGNs began to appear in WT mice from P6 [1]. (D) WT mice (10 months old) showed hearing loss with aging. (E) c-Ret-KI Y1062F/+ -mice, in which the number of Y1062-phosphorylated SGNs was about half of that in WT mice (light blue triangles), suffered from acceleration of age-related hearing loss with decreased cell density of SGNs without functional and morphological abnormalities of hair cells [18]. Acceleration of age-related hearing loss in c-Ret-KI Y1062F/+ -mice was rescued by introducing constitutively activated RET [18].
Our recent in vivo studies suggested that decreased levels of phosphorylated Y1062 in c-RET reduce Akt and NF-κB activities in SGNs [1, 18] (summarized in Figure 2). Furthermore, both congenital and age-related hearing losses with partial impairment of NF-κB in SGNs from c-Ret-KI Y1062F mice were rescued by introducing constitutively activated RET [1, 18]. A previous study demonstrated that mice with impaired function of NF-κB exhibited acceleration of age-related auditory nerve degeneration, resulting in age-related deafness [33]. Thus, these results suggest that impairments of the c-Ret-mediated signaling pathway involve decreased activities of Akt and NF-κB signaling, resulting in degeneration of SGNs.

**c-RET-mediated hearing loss in humans**

The results obtained with c-Ret-KI mice showed that impairments of Y1062 phosphorylation in c-Ret cause early- and late-onset of hearing losses, while normal hearing is maintained with even complete impairment of S697 phosphorylation in c-Ret [1, 18]. In humans, we found hearing loss in three patients with severe HSCR, including two males with homozygous and heterozygous mutations at arginine 969 located in c-RET kinase domains and one male with a nucleotide deletion at RET codon 13 resulting in a frameshift and termination at codon 22. Meanwhile, no hearing impairments were found in patients with other heterozygous missense mutations at codons 30, 144, 489, 734, 897 and 942 [1]. Therefore, these results suggest that impairments of c-Ret and c-RET site-dependently cause hearing losses in mice and humans. Further study is needed to determine the correlation between mutational sites of c-Ret and c-RET and hearing losses in mice and humans.

**Conclusions**

Our studies provide direct evidence that c-Ret is a novel deafness-related molecule in humans and mice. These studies indicated the importance of considering the activity as well as the expression of the target molecule in order to elucidate the etiologies of hereditary deafness. The findings will be useful for the development of new diagnostic and therapeutic strategies targeting c-RET kinase against hearing losses.

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References


