Hes1: a key role in stemness, metastasis and multidrug resistance

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Abbreviations: CSCs, cancer stem cells; EMT, epithelial–mesenchymal transition; MASH-1, Mammalian achaete-scute homolog-1; bHLH, basic helix-loop-helix; TLE, transducin-like Enhancer of split; HDACs, histone deacetylases; dnMAM, dominant-negative mastermind; Runx2, Runt-related protein 2; ABC, ATP-binding cassette; NICD, Notch intracellular domain; CSL, CBF1/ Suppressor of Hairless / Lag1; MAML, Mastermind-like protein family; GSI, γ -secretase inhibitor.

Hes1 is one mammalian counterpart of the Hairy and Enhancer of split proteins that play a critical role in many physiological processes including cellular differentiation, cell cycle arrest, apoptosis and self-renewal ability. Recent studies have shown that Hes1 functions in the maintenance of cancer stem cells (CSCs), metastasis and antagonizing drug-induced apoptosis. Pathways that are involved in the up-regulation of Hes1 level canonically or non-canonically, such as the Hedgehog, Wnt and hypoxia pathways are frequently aberrant in cancer cells. Here, we summarize the recent data supporting the idea that Hes1 may have an important function in the maintenance of cancer stem cells self-renewal, cancer metastasis, and epithelial–mesenchymal transition (EMT) process induction, as well as chemotherapy resistance, and conclude with the possible mechanisms by which Hes1 functions have their effect, as well as their crosstalk with other carcinogenic signaling pathways.

Introduction

Since the concept of stem cells has been implicated in cancer, it has taken many decades to increase the understanding of the biological properties of CSCs, which are characterized by a remarkable ability for extensive proliferation, self-renewal, invasion, development of distant metastasis, and drug resistance.^{1,2} With the efforts to investigate CSCs biology, some of the molecular components that sustain CSC properties are being uncovered. One of these is the Hes1 protein. Notably, the level of Hes1 is a key signature that maintains stem cells, quiescent cells or cancer stem cells in a non-dividing state.^{3–5} Apart from this, Hes1 has additional cellular functions that have not been previously epitomized. Hes1 also contributes to tumor multidrug resistance as well as metastasis.

In this review, we discuss both the significance of Hes1 function in tumors and CSCs and the mechanisms by which Hes1

contributes to stemness, metastasis and drug resistance. Elucidating the pathways involved in the control of Hes1 expression could provide valuable information for cancer treatment.

Overview of Hes1 Factor

Early described as a transcriptional inhibitor, Hes1, which can antagonize neuronal cell fate regulators, such as Mammalian achaete-scute homolog-1 (MASH-1 in the mouse and HASH-1 in humans), Math, and neurogenin (Ngn), is a member of the basic helix-loop-helix (bHLH) family.^{6–11} Hes1 factors is identified with 3 conserved domains: the bHLH, Orange and WRPW domains. The bHLH domain of Hes1 is responsible for interaction with lineage-specific bHLH factors; for instance, it can form a functional heterodimer with other bHLH factors, such as E47, Id proteins as well as its homologues hey1/2.^{12,13–15} Within the bHLH region, Hes1 has a proline residue which exhibits unique binding ability to N box (CACNAG), differing from other bHLH factors which have higher affinity to E box (CANNTG).¹¹ The orange domain is less conserved and confers selection specificity of bHLH heterodimer partner.^{11,16} Another functional region, the WRPW domain, is characterized with the C-terminal Trp–Arg–Pro–Trp sequence.

There are 2 possible mechanisms that Hes1 promote transcriptional repression. Hes1 forms a non-DNA binding complex with other bHLH factors through its bHLH domain. Second mechanism is active repression. Hes1 binds to N box by forming complexes with co-repressor transducin-like enhancer of split (TLE), homologs of *Drosophila* Groucho via WRPW motif.^{17,18} The transcriptional inhibition is mediated by this interaction, and occurs by recruitment of histone deacetylases (HDACs).^{3, 19} In addition, Hes1 may recruit HDACs using the bHLH domain to repress transcription.^{12,20}

However, recent studies show that Hes1 is more than a repressor. Hes1 amplifies Runx2 expression by cooperating with pRb, and WRPW domain is necessary for Hes1–pRb interaction.⁴² In addition, Hes1 uses bHLH domain and orange domain to bind with STAT3 and activates STAT3 phosphorylation.⁵⁸

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The role of Hes1 in CSCs maintenance

Hes1 is widely expressed in different tissue and cell types, including neuronal stem cells, embryonic stem cells, quiescent cells, and especially in cells at the precursor stage. Hes1-deficient mice have been shown to exhibit premature differentiation and rigorous defects in brain tissue.⁶ The overexpression of Hes1 inhibits neuronal and glial differentiation, and the cells most likely maintain their self-renewal ability, if Hes1 is up-regulated.⁷ Furthermore, the undifferentiated state can be sustained in quiescent cells, endodermal endocrine cells and intestine progenitor cells through Hes1 overexpression.⁸⁻¹⁰ Hes1 plays an essential role in stem cells in the undifferentiated state due to its repression ability.

Many tumors, which are composed of a heterogeneous group of cells, have recently been identified as having a small population of cells, also called cancer stem-like cells, which have a high level of stem cell-like properties, including an undifferentiated state, resistance to cell death, self-renewal and tumorigenesis. Considering the tremendous potential that Hes1 has in maintaining self-renewal ability, it was important to investigate the significance of roles these factors play in CSC. Neuroblastoma, originating from the sympathetic nervous system, has embryonic features. The suppression of Hes1 leads to HASH-1 upregulation, which impairs the neuroblastoma undifferentiated state.²¹ The level of Hes1 in CD133⁺ glioma, a tumor of the brain, is elevated. Strikingly, after shRNAs against Hes1 are applied, the colony-forming ability of CD133⁺ glioma is reduced.²² In addition, when the Hes1 level reduces, CD133⁺ positive cells are depleted.²³ Meanwhile, CD133 is identified as a marker of cancer stem cells.²⁴ The mis-expression of Hes1 decreases the tumor sphere-forming capacity and self-renewal ability of oral squamous cell carcinoma cells.²⁵ The same phenomenon can be observed in colon cancer, as well as pancreatic cancer.^{26,27} The Notch-Hes1 pathway also functions significantly in maintaining breast cancer stem cells.²⁸ During a leukemia blast crisis, patient samples that were tested exhibited elevated levels of Hes1.²⁹ Hes1 may promote tumor cell growth and the self-renewal phenotype under hypoxia conditions or Notch stimulation.^{30,31} Downstream factors of Hes1, which tend to be differentiating makers, such as Mash1 and NeuroD show dose- and time-dependent response to Hes1 inhibition treatment in cancer progression, confirming the idea that Hes1 maintains the undifferentiated state, possibly through transcriptional repression.^{21,32,33}

Hes1 contributes to cancer metastasis

Tumor metastasis, which occurs in a multistep biological process, wildly spreads cancer cells from a primary tumor to a distant location and indicates a far worse prognosis and increases the risk of death in cancer patients. Importantly, the correlation between Hes1 and tumor metastasis has been revealed through a series of experimental investigation.

The accumulating experimental data indicate that a malignant tumor tending to metastasizes is often accompanied by aberrant Notch signaling and the elevation of Hes1.³⁴⁻³⁷ Hes1 loss of function via a dominant-negative mastermind (dnMAM) in an osteosarcoma xenograft model, which then results in a significant

reduction of lung metastasis neoplasm, while dnMAM has no effect on tumor latency or tumor growth rates.³⁸ Evidence from 56 samples obtained from patients who did well with osteosarcoma and died from metastasis reveals that Hes1 is inversely correlated with survival.³⁷ The metastasis potentiality of Hes1 was further studied *in vitro* by constructing mutant Hes1 fragments which were then transfected into osteosarcoma cells. The results suggest that Hes1 represses Deltex1 which can block osteosarcoma invasiveness by binding to the Deltex1 promoter.³⁹

There is yet another mechanism by which Hes1 is able to promote tumor metastasis. Hes1 has been implicated in a tumor cell “osteoblast-like” phenotype and tumor bone metastasis. Runt-related protein 2 (Runx2), also known as Cbfa1, has been well documented to be highly expressed in breast tumors and prostate tumors, as well as their secondary tumor sites. Runx2 plays a critical role in tumor bone metastasis, as discussed by Pratap J.⁴⁰ Interestingly, Hes1 can eliminate the repressive potentiality that the TLE protein exerts on Runx2 by forming a Hes1-Runx2 complex and enhancing the transactivation of Runx2.⁴¹ Additionally, Hes1 increases Runx2-DNA binding, stimulates Runx2 transcriptional activity by increasing the stability of Runx2, and associates with pRb synergistically to augment Runx2 transcription.^{42,43} When examined in prostate tumor bone metastasis, Runx2 DNA-binding activity is enhanced in response to osteogenic induction and is inhibited by Notch-Hes1 inhibition, verifying that Hes1-Runx2 signaling functions critically in tumor bone metastasis.⁴⁴

Furthermore, EMT process allows the loss of junction with adjacent cells, the polarization of epithelial cells and subsequent acquisition of mesenchymal features. During EMT, cells undergo multiple biologic changes including enhanced motility capacity, invasiveness and resistance to apoptosis has been well established to have an intimate connection with tumor metastasis and also shows its deep relationship to cancer stem cells.^{45,46} In recent investigations, Hes1 has been found to potentially act as a regulator of EMT, inducing phenotype plasticity.

To initiate EMT, glioma cells lose their phenotype and cell-cell adhesion and become invasive through β -catenin and NF- κ B synergistically, a process that is enhanced by Notch1 via AKT signaling.⁴⁷ Previous studies on Notch and AKT signaling suggest that Notch signaling can stimulate the AKT pathway by suppressing PTEN levels.^{48,49} Interestingly, Hes1 can effectively abrogate PTEN by binding to its promoter, and when shRNA silence of Hes1 is performed, PTEN transcription shows a moderate increase.^{48,50} If transfected with Hes1 siRNA, clear cell renal cell carcinoma cells have a significant change in PTEN level and reduced invasiveness.⁵¹ In addition, Hes1 is required to promote Snail-dependent EMT in HK-2 cells that are cultured in high glucose conditions.⁵²

Hes1 leads to tumor multidrug resistance

Drug resistance is a major cause of tumor treatment failure and cancer recurrence. Based on the “cancer stem cell” theory, the small population of pluripotent cells develops a range of strategies to promote chemotherapy insensitivity, including ATP-binding cassette (ABC) transporter expression, DNA repair

capacity and quiescence.^{53,54} This may provide a new avenue to target CSCs, as the Hes1 anti-drug effect has been recently discovered.

The overexpression of Hes1 antagonizes a pro-apoptosis state of myeloma cells.⁵⁵ Correspondingly, the Hes1 level is observed to be higher in chemically resistant, as well as cyclophosphamide-treated hepatocellular carcinoma.⁵⁶ There are several latent mechanisms by which cancer cells are able to promote transformations that increase drug resistance. Such instances include high level of Hes1 expression and enhancing STAT3 phosphorylation.⁵⁷ On the other hand, the modulation of STAT3 phosphorylation is affected by Hes1 binding to STAT3 directly and then recruiting JAK and Src protein effectively.^{58,59}

Tumor dormancy appears to be the underlying mechanism for tumor resistance to chemotherapy. Hes1 correlates with dormancy, and this is mediated, in part, by retaining the tumor in a quiescent stage that can re-enter the cell cycle.^{3,60-62} Indeed, it should be noted that cancer cell dormancy resulting from cell cycle arrest has been observed in solid tumors, while the quiescent stage is reached by several pathways, in addition to cell cycle arrest.⁶³ Experiments *in vitro* have shown the involvement of Notch receptor, Hes1, and p21 activities in the drug resistance of quiescent tumors. Notch1 activates the p21-induced cell-cycle-arrest tumor model, which is identified as a drug-resistant phenotype.^{64,65} However, sustained activation of Notch-p21 contributes to an irreversible senescent phenotype.^{8,66} In contrast, cells that express full-length Hes1 genes resume proliferation, after a p21-induced senescent phenotype, as Hes1 transcriptionally represses p21 in a bHLH domain-dependent manner and Hes1 keeps quiescent cells from the differentiation state mentioned previously.^{8,67} Based on this, we may conclude that the quiescence-based dormancy relies on the balance between Hes1 and p21.

As seen below, we conclude that Hes1 plays an extraordinarily important role in tumor progression (Fig. 1).

Control of Hes1 expression

The mechanism that leads to an induction of Hes1 cancer cells could possibly represent a promising therapeutic target. In this section, we have only highlighted some signaling pathways that have been implicated in the induction of Hes1. We will discuss what is currently known about the mechanism by which they up regulate Hes1 (Fig. 2).

Figure 1. The role of Hes1 in tumor progression. Hes1 contributes to CSCs maintenance, cancer chemotherapy resistance and tumor metastasis mainly through its downstream protein.

Canonical Notch signaling pathway

The Notch signaling pathway in mammals consists of 5 transmembrane ligands (Delta-like 1, 3 and 4 and Jagged 1 and 2) and 4 membrane bound receptors (Notch 1, 2, 3 and 4). Following ligand binding, the Notch receptor undergoes the second cleavage mediated by ADAM/TACE, and the third proteolytic cleavage catalyzed by γ -secretase and releases the Notch intracellular domain (NICD), which translocates to the nucleus and then associates with DNA-binding factors CSL (CBF1/ Suppressor of Hairless / Lag1) and the Mastermind-like protein family (MAML), activating Hes family transcription.⁶⁻¹¹

The expression of Hes1 induced by Notch can be detected more than embryo neural stem cells, intestinal crypt progenitor cells, pancreatic stem cells, bone marrow mesenchymal progenitors and quiescent skeletal muscle stem cells.^{9,10,68-71} Treatment with γ -secretase inhibitor (GSI) results in the suppression of Hes1 and the differentiating state of those stem cells, indicating the role of the Notch pathway in Hes1 expression, as well as the essential status of the Notch-Hes1 pathway in facilitating cell fate decisions and regulating differentiation during development.

Indeed, the upregulation of Hes1 is involved in maintaining stemness in mammals and reflects elevated Notch signaling pathway activity, which may contribute to the growth of those tumors. Aberrant Notch-Hes1 axis is presented in cancer stem cells, such as glioblastoma, breast cancer, pancreatic cancer, and osteosarcoma.^{27,36,72,73} The inhibition of the Notch signaling pathway can reverse cancer cell survival and induce apoptosis. In glioma, the up-regulation of Notch enhances Nestin and CD133 expression.^{23,74} After Notch is blocked by GSI, we observe a population of cancer stem cell depletion.²³ Aberrant Notch-Hes1 pathway is observed in invasive cells which undergo EMT process.⁴⁸⁻⁵¹ In dormant cancer cells, Notch-Hes1 activation can be

Figure 2. Signaling pathways that modulate the level of Hes1.

detected.^{64,65} Above all, the Notch-Hes1 signaling pathway may play a functional role in self-renewal, CSCs maintenance and tumor progression.

Furthermore, crosstalk has been implicated between canonical Notch, Wnt, and hypoxia signaling. β -catenin, a downstream factor of Wnt signaling, increases Hes1 expression by binding with NICD and stabilizes the CSL-NICD complex by recruiting p300 and P/CAF.⁷⁵⁻⁷⁷ HIF-1 is required to promote interactions with NICD and potentiate the stabilization of the CSL-NICD complex under hypoxia conditions, while induction of Hes1 protects progenitor cells, as well as tumor stem cells, against differentiation.⁷⁸⁻⁸¹

Non-canonical Notch signaling pathway

Notch signaling pathway can exert its biological function independently of its ligands, receptors or CSL in vertebrates. Early evidence for non-canonical Notch has been implicated in normal tissue as well as tumor.^{82,83} The downstream Notch target gene Hes1 is modulated in a Notch independent manner as well.

Hes1 upregulation mediated by Hedgehog signaling promotes stem/glia cell undifferentiated state in ventricular zone and is not influenced by CSL deletion.⁸⁴ Hes1 inhibition which is conducted by cyclopamine incubation indicates that Hes1 expression involves canonical Hedgehog signaling, because cyclopamine blocks Hedgehog downstream factor Smo function.⁸⁵ In the retina progenitor cells, Hedgehog signaling activates Hes1 expression in the presence of GSI, as Hedgehog downstream factor Gli2 directly binds to Hes1 promoter and accelerate Hes1 level without activating CSL.⁸⁶ The requirement of Gli2 in Hes1 regulation is also observed in telencephalic neuroepithelial stem cells.⁸⁷ Gli1 also shows its regulation on Hes1. In neural stem cells, overexpression of Gli1 is accompanied with Hes1 upregulation.⁸⁸ In addition, the Hedgehog-Hes1 pathway can be detected in tumor cells as well. To initiate tumor or maintain undifferentiated properties, Hes1 induction mediated by Hedgehog pathway can be detected during tumorigenesis.⁸⁹ Interestingly, Hes1

inhibits Hedgehog signaling by binding to the first intron of Gli1 in brain, prostate, lung, ovarian, skin, and hematopoietic cell cancers.⁹⁰ This may suggest a potential chemotherapy resistance mechanism that tumor survives long term Notch-Hes1 inhibition with Gli1 rescuing expression.

Furthermore, other signaling pathways have involved in non-canonical Notch pathway. FGF2 activates c-Jun N-terminal kinase (JNK) in neural progenitors, and the activated JNK further phosphorylates ATF2. Phospho-ATF2 binds to the Hes1 promoter

to enhance Hes1 expression and high level of Hes1 maintains a neural progenitor cells pool.⁹¹ In the TGF α -stimulated neuroblastoma, Hes1 upregulation follows ERK1/2 phosphorylation without NICD activation and low level of HASH-1 suppressed by Hes1 contributes to maintain neuroblastoma embryonic non-dividing states and proliferative capacity.⁹²

Conclusion

Several therapeutic avenues to reverse aberrant Notch and non-canonical Notch have been applied in researches. Since Hes1 lies at the crossroads of multiple signaling pathways, co-inhibition of these pathways might represent a new strategy for cancer therapy. For instance, when treated with anti-hedgehog and anti-Notch compounds *in vitro*, glioma cells show apoptosis phenotype, CSCs depletion and sensitivity to temozolomide.^{90,93} As discussed earlier, the third cleavage mediated by γ -secretase is a crucial step for Notch activation and is targeted by GSI inhibitors which have been evaluated in phase 1 clinical trials, such as MK-0752, PF-03084014, RO-4929097, reviewed by Takebe N.⁹⁴ With the rigorous estimation in preclinical models, it is safe to say that GSI inhibitors do show its anti-proliferation, anti-CSC and pro-apoptotic effects on tumor. However, GSI inhibits Notch target genes without selection which causes a rapid differentiation of intestinal progenitor cells into goblet cells.⁹⁵ This may be the primary cause of gastrointestinal toxicities associated with GSI. Aiming at Hes1 may result in fewer side effects because many other Notch target genes will be unaffected.

As we have discussed the fundamental role of Hes1 molecule in tumor cells and the strategies used by tumor cells to maintain stemness, metastasize and resist chemotherapy, targeting Hes1 may represent a potential therapeutic possibility to target cancer. Therefore, significant attention should be paid to clinical development to target Hes1.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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