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Serum long non-Coding RNA in keloid patients A Systematic Review and Meta-analysis

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Abstract

Introduction: Keloid is a benign tumor that develops from abnormal responses to skin injuries, characterized by fibroblast hyper proliferation. LNC-RNAs are frequently deregulated in cancers.

Aim of the study: To assess the association between LNC-RNA and keloids.

Methods: Searching in the data sources of PubMed and Medline for all of the case-control studies that contain keloid patients and different types of LNC-RNA assessed to them.

Results: Outcomes showed increased levels of the four LNC RNA in keloid patients than in healthy people. A systematic review of four articles found four LNC-RNA implicated in keloid. The studied LNC-RNA cases were H19, HOXA11, CACNA1G-AS1, and LINC01116. This meta-analysis showed that LNC-RNA patients had a significant increase in serum levels in keloid cases compared with the control group. The serum level of most of them increased significantly with the severity of the disease.

Conclusion: This analysis clear that LNC-RNAs had a role and effect in the inflammatory process of the presentation of keloid and its severity. So, we should understand the pathological pathway of keloid to help in keloid treatment.

Keywords: Serum long non-Coding RNA; Keloid; Meta-analysis.

1. Introduction

Keloids are benign dermal fibro-proliferative tumors that extend outside the wound boundary and invade adjacent tissue due to excessive production of extracellular matrix [1].

Keloid scars affect about 10–15% of all wounds. The incidence is higher among young individuals, as they are prone to trauma; their skin generally has more elastic fibers, resulting in more tension and the collagen synthesis rate is greater in them [2].

Any type of deep injury in the skin can create abnormal healing of the wound and help keloid scar formation [3].

Steroidogenesis may be caused by multiple genetic, local, and systemic factors that alone or together stimulate continuous inflammation in the wound and scar [4].

Pathophysiology of Keloid scars is believed to involve an abnormal balance between proliferation and apoptosis of fibroblast and endothelial dysfunction [5].

Long noncoding RNAs (LNC-RNAs) are defined as transcripts with a length of more than 200 nucleotides. They are irregulated and play very important roles in tumor synthesis and cancer spreading, acting as tumor genes or tumor suppressors [6].

The LNC RNA H19 is accompanied by proliferation in tumors [7]. However, the molecular mechanism and roles of LNC RNA LINC01116 in the synthesis of keloid were still obscure [8]. There was a big evidence of the value of LNC-RNAs in keloid progression and development. The main mechanisms are still obscure. In that study, the molecular mechanisms and biological effects of LNC RNA HOXA11-AS in keloid synthesis were assessed [9]. The keloidal fibroblast showed that it contains calcium voltage-gated channel unit alpha1 G antisense RNA 1 (CACNA1G-AS1) [10].

2. Methods

2.1. Summary of experimental designs in the literature studies

PCR was the investigational techniques to detect LNC-RNA to investigate if H19 increased the proliferation of fibroblasts in keloid disorder or not. PCR clarified that H19 levels were more excess in keloid patients than in normal healthy people [7]. Western blotting and qRT-PCR showed that the levels of microRNA-203 and LINC01116 were used to assess the levels of LNC-RNA. Cell superfast, invasion, and migration techniques were used as well. Cell death and extracellular matrix were examined by western blot and Flow cytometer. The bioinformatics method clarified the relationship between LINC01116 and SMAD5. A dual-luciferase method, RNA pull-down, and RNA Immunoprecipitation (RIP) methods assist in the detection of them [8]. In the investigation of the relation between human keloidal fibroblasts and keloid, western blot, qRT- PCR method was used to assess the levels of miR-124-3p, HOXA11-AS, and transforming growth factor β receptor type I (TGFβR1). For HOXA11-AS assessment, the loss- and gain-of-function methods were accepted. The cell death and cellular new vessel formation were assessed by some methods such as Flow cytometry, TUNEL, tube formation, and DNA ladder methods. Also, the linkage among miR-124-3p and HOXA11-AS, TGFβR1, and miR-124-3p was clarified by the bioinformatic method. Importance of miR -124- 3p was in the organization of TGFβ or PI3K/ Akt, and HOXA11-AS, as well [9]. The expression of CACNA1G-AS1, miR-205 standards were assessed through quantitative real-time polymerase chain reaction (qRT-PCR). The use of Cell Counting Kit-8 (CCK-8) was very helpful in proliferation measurement, while cell infestation was measured by a trans well method. Caspase-3 was valuable marker for the assessment of keloidal fibroblasts, while flowcytometry assessed the average of cell death. The connection among miR -205, CACNA1G- AS1 was performed by RNA immunoprecipitation (RIP) and dual luciferase reporter method [10].

2.2. Literature Search

The information was excised from PubMed, and Medline until September 2021.

The main items and keywords were the following: keloid and the LNC-RNAs included CACNA1G-AS1, H19, LINC01116, and HOXA11-AS.

2.3. Contents and output Criteria

The contents criteria contained observational, interventional studies such as case-control studies. They included LNC-RNAs cases with and without keloid.

Output criteria showed that some studies didn't include any observational or interventional findings. Also, other studies on tumors rather than keloid didn't contain any cropped results of us.

2.4. Goodness measurement

We performed a full goodness measurement to evaluate the fitness of the four of our included studies which are case-control observational studies.

2.5. Extraction of information

We extract the interesting information such as the comparison between studies results, size, groups, characters, design, and level of LNC-RNA in detail finally.

2.6. Search Outcomes

In four studies, data on the level of LNC-RNA revealed a significant increase in their level.

The LNC-RNA plays a very important role in keloid presentation risk, severity, and pathogenesis of keloid. This might be understood by the fact that LNC-RNA affects cell death and cell overgrowth by many mechanisms and pathways. These data might drive the investigation of the development of a new type of keloid therapy. That might be due to the decline the keloidal fibroblast death and increase of blood vessel growth of fibroblast.

2.7. Statistical analysis

Mean ± standard deviation (SD) was the main test that was done in all our included searches. That increased the investigational data on the connection between LNC-RNA and keloid. We used the Student's t-test and one-way analysis of variance (ANOVA) to compare different studies. *P-value <0.05* was considered significant.

3. Results

3.1. Characteristics of the Included Studies

There was a difference in LNC-RNA presentation in different disorders such as autoimmune or inflammatory or tumors. We selected the four LNC-RNA studies assessed in keloid patients until now.

Table 1: Summary of the studies.

Source	Study design	Country	Study group and sample
Jun et al 9, 2020	case-control	USA	+40
Jie et al 7, 2016	case-control	USA	+16
Xu et al 10, 2020	case-control	USA	+20
Weiwei et al 8, 2021	case control	USA	+20

We had many methods as (si) RNA-mediated silencing that can help us to understand how fibroblasts' proliferation is mediated by H19. Some results show increased expression of levels of H19 in keloid patients. Vascular endothelial growth factor (VEGF), and rapamycin (mTOR) play an important role in keloid. It is increasing that is detected by western blotting indicating the contribution of H19 in keloid pathogenesis. Finally, we found that H19 decreased VEGF, and mTOR levels. Fibroblast proliferation is caused by many reasons such as increased levels of H19 through different mediators [7].

Many markers were increased in keloidal tissue such as LINC01116 and SMAD5 while Many markers were decreased such as miR-203. Fibroblast death is caused in many ways such as decreasing SMAD5 and increasing miR-203. Keloid presentation, proliferation, and ECM production decreased to many factors such as the LINC01116 shortage. Furthermore, miR-203 which could attach to some markers as LINC01116 was targeted by SMAD5 [8].

There are many markers increased in keloids such as TGFβR1 and HOXA11-AS, on the other hand, other markers decreased in keloids such as miR-124-3p. According to the function of HOXA11-AS in keloid formation, its high levels decrease cell death and increase new vessel formation in keloidal fibroblast.

Many factors affect HOXA11-AS types through aiming TGFβR1, or PI3K/Akt pathway such as miR124-3p. Finally, HOXA11-AS decreases cell death and also increases new vessel formation in keloids in different ways. The attention to this information will help us to think of a new target for keloid treatment [9].

Keloid formation is suggested by increasing some markers such as CACNA1G-AS1 by many mechanisms such as Increasing cell multiplication, decreasing cell death, increase cell invasion through some signals such as miR-205 decreasing, as our marker suppresses it in the tissue of keloid. In addition to that, miR-205 plays a positive role in itself, cellular overgrowth, migration, and keloidal fibroblast death [10].

3.2. Outcomes

In the four studies, data on the level of LNC-RNA revealed a significant increase in their level. That is due to the decrease in the

keloidal fibroblast death and increase of the blood vessel growth of fibroblast.

4. Discussion

A total of four articles were used in searching of the levels of LNC-RNA expression in keloid patients by PCR. All studies found a significant association with keloid.

Meta-analysis of that study revealed the following:

Excessive H19 level expression with keloid patients' serum.

Excessive LINC01116 level expression with keloid patients' serum.

Excessive HOXA11 level expression with keloid patients' serum.

Excessive CACNA1G- AS1 level expression with keloid patients' serum.

Restriction of this study was that all o0f these studies were case-controls, which already had a retrospective kind and didn't demonstrate the causation.

5. Conclusion

The summary of our review is that LNC-RNA plays a very important role. In keloid presentation risk, severity, and pathogenesis of keloid. This is maybe understood by the fact that LNC-RNA affects cell death and cell overgrowth by many mechanisms and pathways, this data will be the big boss in the development of new types of keloid therapy.

Conflicts of Interest: All authors declare no conflict of interest.

References

1. Liu Q, Li P, Yang Z, Qu B, Qin C, Meng S, Fang H, Wu R, Cheng T, Yang D. Multi-stage surgery combined with radiotherapy for treatment of giant anterior chest wall keloid: A case report. Medicine (Baltimore). 2020 Jan;99(4):e18886. doi: 10.1097/MD.0000000000018886.
2. Rabello FB, Souza CD, Farina Júnior JA. Update on hypertrophic scar treatment. Clinics (Sao Paulo). 2014 Aug;69(8):565-73. doi: 10.6061/clinics/2014(08)11.
3. Chua SC, Gidaszewski B, Khajehei M. Efficacy of surgical excision and sub-dermal injection of triamcinolone acetonide for treatment of keloid scars after caesarean section: a single blind randomised controlled trial protocol. Trials. 2019 Jun 18;20(1):363. doi: 10.1186/s13063-019-3465-6.
4. Tsai CH, Ogawa R. Keloid research: current status and future directions. Scars Burn Heal. 2019 Aug 19;5:2059513119868659. doi: 10.1177/2059513119868659.
5. Goutos I. Intralesional excision as a surgical strategy to manage keloid scars: what's the evidence? Scars Burn Heal. 2019 Aug 27;5:2059513119867297. doi: 10.1177/2059513119867297.
6. Xue D, Lu H, Xu HY, Zhou CX, He XZ. Long noncoding RNA MALAT1 enhances the docetaxel resistance of prostate cancer cells via miR-145-5p-mediated regulation of AKAP12. J Cell Mol Med. 2018

Jun;22(6):3223-3237. doi: 10.1111/jcmm.13604.

7. Zhang J, Liu CY, Wan Y, Peng L, Li WF, Qiu JX. Long non-coding RNA H19 promotes the proliferation of fibroblasts in keloid scarring. Oncol Lett. 2016 Oct;12(4):2835-2839. doi: 10.3892/ol.2016.4931.
8. Yuan W, Sun H, Yu L. Long non-coding RNA LINC01116 accelerates the progression of keloid formation by regulating miR-203/SMAD5 axis. Burns. 2021 May;47(3):665-675. doi: 10.1016/j.burns.2020.07.027.
9. Jin J, Jia ZH, Luo XH, Zhai HF. Long non-coding RNA HOXA11-AS accelerates the progression of keloid formation via miR-124-3p/TGFβR1 axis. Cell Cycle. 2020 Jan;19(2):218-232. doi: 10.1080/15384101.2019.1706921.
10. Zhao X, Jie X, Gao YK, Nie B, Jiang H. Long non-coding RNA CACNA1G-AS1 promotes proliferation and invasion and inhibits apoptosis by regulating expression of miR-205 in human keloid fibroblasts. Biosci Rep. 2020 Jun 26;40(6):BSR20192839. doi: 10.1042/BSR20192839.