HER2 and p53 Expression in Marjolin’s Ulcer

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Abstract

Background: Marjolin's ulcer is a malignant tumor that is different from other skin ulcers, and its pathogenesis is not yet clear. The diagnosis and prognosis of Marjolin's ulcer lack valuable marks on immunohistochemistry (IHC).

Methods: In this study, we detected the expression of HER2 and p53 in Majorlin's ulcer with immunohistochemistry, and retrospectively analyze the clinicopathological characteristics of Marjolin's ulcer samples.

Results: Our results showed that no HER2 but p53 was detected in Majorlin's Ulcer samples. Meanwhile, by statistic analysis we found that the positive expression rate of p53 in Majorlin's Ulcer samples was associated with sex, course of disease, ulcer size, pathological type of ulcer cancreration, and degree of tumor differentiation. Furthermore, we showed that the positive rate of p53 was proportional to the malignancy degree of Marjolin's ulcer.

Conclusions: Our results of this study will lay a foundation for diagnosis of Marjolin's ulcer and even prevention of Marjolin's ulcer progression.

Introduction

Marjolin's ulcer is described as malignant lesions developed in the epithelium of burn scars (1–2). It is first defined that the malignant changes developed on the basis of scar tissue in 1828 by French physician Jean Nicholas Marjolin in a report (3–4). In 1907, Fordyce firstly named these malignant ulcers of skins as Marjolin's ulcer (5). Later, Marjolin's ulcer is termed as different chronic wounds including bum, traumatic wounds, bedsores and diabetes which can transform into malignancies (6). The incidence of Marjolin's ulcers has been reported to be 1% – 2% in old burn scars and male is dominant (7). Notably, the common malignancies which are originated from Marjolin's ulcers include squamous cell carcinoma (SCC), basal cell carcinoma (BCC) and malignant melanoma and sarcoma (8–11). The pathogenesis of Marjolin's ulcer is not yet fully understood. The diagnosis and prognosis of Marjolin's ulcer lack valuable marks.

HER2, a non-ligand-binding member of epidermal growth factor receptor (EGFR) family, exerts its function through heterodimerisation with other EGFR family members. HER2 functionally promotes oncogenesis, which has been best characterised in breast cancer. Recently, HER2 gene mutations have been detected in multiple solid cancers types (12–14). p53 is able to transform cells led to the preliminary impression that p53 was a tumor suppressor gene. However, lots of reports around the world help us understand all aspects of p53 functions: from it being a unstable transcription factor that regulates target genes indispensable for tumour suppression, to to over-expression in several cancer types (15–17).

To explore the expression of p53 and HER2 in Marjolin's ulcer, we collected 106 clinicopathological cases with Marjolin's ulcers who were admitted by Wuhan Third Hospital from 2015 to 2019. Meanwhile, to better understand the regularity of p53 and HER2 occurrence in the context of Majorlin's ulcer, the clinicopathological characteristics of Majorlin's ulcer samples were analyzed.

Materials And Methods

Clinicopathological samples and data. 339 patients with chronic skin ulcer were collected from January 2016 to December 2020 in the Wuhan Third Hospital, including 106 cases of Marjolin's ulcer and 233 cases of non malignant chronic skin ulcer. The clinicopathological characteristics of patients with Marjolin's ulcer and non-malignant chronic ulcer were collected and statistically analyzed, including age, sex, ulcer size, lesion location, course of disease, pathological type of ulcer cancreration, degree of tumor differentiation, depth of tumor invasion, prognosis, and expression of p53 and HER2 in the whole slides of the tumor of Marjolin's ulcer.

Immunohistochemical assay. The surgical specimens of 106 patients with Marjolin's ulcer were fixated in 4% formalin for 24 hours and embedded in paraffin after dehydration. Immunohistochemical assay was performed by EnVision FLEX
immunohistochemistry kit (DAKO, Denmark) according to protocol. Next, samples were incubated with anti-HER2 rabbit monoclonal antibody (Abclonal, China) and anti-p53 mouse monoclonal antibody (Abclonal, China) for 1h at 37°C. After washed three times with phosphate buffer saline (PBS), the sections were incubated with rabbit or mouse anti-IgG antibody for 30 min at 37°C. Washed three times with PBS, samples were developed with diaminobenzidine and counterstained with hematoxylin. The positive expression of HER2 and p53 (brown in the cytoplasm/cell membrane) in tissues was observed with BX51 bio-optical microscope (Olympus, Japan).

Immunostaining results of p53 were evaluated according to Thanaa El.A: 0: negative, 1+: <10% positive cells, 2+: 10–50% positive cells and 3+: >50% positive cells. 0 and 1 + were considered negative (18).

The positive sign of HER2 was described as complete expression in the cell membrane. The expression of HER2 was evaluated according to guidelines of ASCO/CAP. The expression of HER2 was described as ‘negative’ (score 0 or 1, where 0 is no staining or membrane staining in < 10% of tumour cells, and 1 is faint/barely perceptible membrane staining in > 10% of tumour cells, with cells only stained in part of their membrane), ‘equivocal’ (score 2, weak to moderate complete membrane staining in > 10% of tumour cells) or ‘strongly positive’ (score 3, strong complete cell membrane staining in > 10% of the tumour cells).

**Statistical analysis.** The data were statistically analyzed by SPSS 22.0 statistical analysis software. Clinicopathological data with Marjolin's ulcer and non-malignant chronic ulcer was compared and analyzed by the Pearson chi-square test. \( P < 0.05 \) was considered significant.

**Results**

*Characteristics of Marjolin's ulcer canceration.* To better understand the characteristics of Marjolin's ulcer samples, Marjolin's Ulcer was sorted by canceration level including squamous cell carcinoma, basal cell carcinomas, sarcoma and malignant melanoma (Fig. 1). Furthermore, the frequency of pathological types and invasion depth of Marjolin's ulcer canceration was analyzed. As shown in Table 1, The SCC was the most common type in the patients of Marjolin's ulcer (86.7%). Among of SCC, high differentiation was primary compared to moderate and poor differentiation. Other pathological types of ulcer canceration were basal cell carcinomas (7.5%), malignant melanoma (4.7%) and sarcoma (0.9%)(Table 1). Moreover, the depth of tumor invasion mainly reached dermis (89.6%), followed by bone and muscle (10.4%)(Table 1).
Table 1
Frequency of pathological types and invasion depth of Marjolin’s ulcer canceration

| Pathological types and invasion depth | Frequency, n(%) |
|--------------------------------------|-----------------|
| Pathological types                   |                 |
| Squamous cell carcinoma              |                 |
| High differentiation                 | 51(48.1)        |
| Moderate differentiation             | 33(31.1)        |
| Poor differentiation                 | 8(7.5)          |
| Basal cell carcinomas                | 8(7.5)          |
| Malignant melanoma                   | 5(4.7)          |
| Sarcoma and others                   | 1(0.9)          |
| Depth of tumor invasion              |                 |
| Dermis                               | 95(89.6)        |
| Bone and muscle                      | 11(10.4)        |

Expression of HER2 and p53 in Marjolin’s ulcer. To explore whether Marjolin’s ulcer expressed HER2 and p53, Marjolin’s ulcer samples were detected by immunohistochemical assay with anti-HER2 and anti-p53 antibody respectively. As result shown, all patients in the Marjolin’s ulcer group had negative expression of HER2 (Fig. 2). Interestingly, we found p53 was detected in Marjolin’s ulcer samples (Fig. 3). The positive expression rate of p53 was 56.6% in Marjolin’s ulcer totally (Table 2).

Clinicopathological characteristics of Majorlin’s ulcer samples. To better understand the regularity of p53 occurrence in the context of Majorlin’s ulcer, we explore the relationship of p53 expression with clinicopathological characteristics of Majorlin’s ulcer samples. As shown in Table 2, male patients had lower positive expression rate of p53 than female patients (41.3% vs. 79.1%, $\chi^2 = 14.866, P < 0.05$). Furthermore, the positive expression rate of p53 was 63.3% for patients with course over 20 years, none of patients with course between 10 and 20 years, and 52.6% for patients with course less than 5 years ($\chi^2 = 11.995, P < 0.05$). The positive expression rates of p53 with ulcer area less than 2 cm, in 2.1-5 cm, and over 5 cm were respectively 75.0%, 44.6%, and 76.2% ($\chi^2 = 9.838, P < 0.05$). The positive expression rates of p53 were 53.3%, 62.5%, 100%, and 0 for patients with SCC, basal cell carcinoma, malignant melanoma, and sarcoma, respectively ($\chi^2 = 20.172, P < 0.05$), which indicated that the degree of malignancy of Marjolin’s ulcer was proportional to the positive expression rate of p53. The positive expression rates of p53 were 35.3%, 75.6%, and 75.0% for well-differentiated SCC, moderately differentiated SCC, and poorly differentiated SCC ($\chi^2 = 14.841, P < 0.05$). Hence, the positive rate of p53 was associated with sex, course of disease, ulcer size, pathological type of ulcer canceration, and degree of tumor differentiation, and there was no significant difference in the positive expression rates of p53 between patients with different age, etiologies, lesion location, depth of tumor invasion, and prognosis (Table 2).
Table 2
Expression of p53 in the patients with Marjolin’s ulcer.

| Clinical characteristics | Group | Number of samples (n) | ++ | ++ | - | Positive expression rate | $\chi^2$ value and P value |
|--------------------------|-------|-----------------------|----|----|---|---------------------------|---------------------------|
| Sex                      | male  | 63                    | 10 | 16 | 37 | 41.3%                     | $\chi^2 = 14.866, P < 0.05$ |
|                          | female| 43                    | 21 | 13 | 9  | 79.1%                     |                           |
| Age (years)              | 0–10  | 0                     | 0  | 0  | 0  | 0                         | $\chi^2 = 3.346, P > 0.05$ |
|                          | 11–20 | 2                     | 0  | 0  | 2  | 0                         |                           |
|                          | 21–30 | 12                    | 4  | 2  | 6  | 50%                       |                           |
|                          | 31–40 |                       |    |    |    |                           |                           |
|                          | 41–50 | 59                    | 18 | 18 | 23 | 61.0%                     |                           |
|                          | 51–60 |                       |    |    |    |                           |                           |
|                          | 61–70 | 33                    | 9  | 9  | 15 | 54.5%                     |                           |
|                          | 71–80 |                       |    |    |    |                           |                           |
|                          | 81–90 |                       |    |    |    |                           |                           |
|                          | >90   |                       |    |    |    |                           |                           |
| Course of disease (years)| <5   | 19                    | 10 | 0  | 9  | 52.6%                     | $\chi^2 = 11.995, P < 0.05$ |
|                          | 5–10 | 0                     | 0  | 0  | 0  | 0                         |                           |
|                          | 10–20| 0                     | 0  | 0  | 8  | 0                         |                           |
|                          | >20  | 79                    | 21 | 29 | 29 | 63.3%                     |                           |
| Etiologies               | Burn | 92                    | 25 | 26 | 41 | 55.4%                     | $\chi^2 = 2.481, P > 0.05$ |
|                          | Bedsore | 6                  | 2  | 1  | 3  | 50%                       |                           |
|                          | Diabetes | 5                 | 2  | 1  | 2  | 60%                       |                           |
|                          | Trauma  | 2                   | 1  | 1  | 0  | 100%                      |                           |
|                          | Other  | 1                   | 1  | 0  | 0  | 100%                      |                           |
| Ulcer size (cm)          | 0–2  | 20                    | 10 | 5  | 5  | 75%                       | $\chi^2 = 9.838, P < 0.05$ |
|                          | 2.1–5| 65                    | 10 | 19 | 36 | 44.6%                     |                           |
|                          | >5   | 21                    | 11 | 5  | 5  | 76.2%                     |                           |
| Lesion location          | Head and neck region | 34    | 20 | 2  | 12 | 64.7%                     | $\chi^2 = 5.658, P > 0.05$ |
|                          | Upper limbs | 19   | 8  | 5  | 6  | 68.4%                     |                           |
|                          | Trunk | 5                    | 0  | 1  | 4  | 20%                       |                           |
|                          | Lower limbs | 48 | 3  | 21 | 24 | 50%                       |                           |
| Prognosis                | Recurrence | 19 | 7  | 4  | 8  | 57.9%                     | $\chi^2 = 4.456, $ |
Clinical characteristics | Group | Number of samples (n) | ++ | ++ | - | Positive expression rate | P > 0.05 χ² value and P value
--- | --- | --- | --- | --- | --- | --- | ---
Death | 5 | 4 | 0 | 1 | 80% | | |
| Lose | 9 | 4 | 2 | 3 | 66.7% | | |
| Cure | 56 | 7 | 20 | 29 | 48.2% | | |
| Not delay | 17 | 9 | 3 | 5 | 70.6% | | |

Pathological types of ulcer canceration | Degree of tumor differentiation | Well-differentiation | 51 | 3 | 15 | 33 | 35.3% | χ² = 14.841, P < 0.05
| Moderate differentiation | 33 | 19 | 6 | 8 | 75.6% | | |
| Poor differentiation | 8 | 5 | 1 | 2 | 75% | | |
| Basal cell carcinomas | 8 | 2 | 3 | 3 | 62.5% | | |
| Malignant melanoma | 5 | 3 | 2 | 0 | 100% | | |
| Sarcoma and others | 1 | 0 | 0 | 1 | 0 | | |

Depth of tumor invasion | Dermis | 95 | 24 | 28 | 43 | 54.7% | χ² = 1.299, P > 0.05
| Bone and muscle | 11 | 7 | 1 | 3 | 72.7% |

Discussion

Marjolin’s ulcer, mainly caused by burn scars, is relatively rare (19–20). However, in this study we found that there were 106 (31.3%) with Marjolin’s ulcer in 339 patients with cutaneous chronic ulcers treated in our hospital. Malignant changes arising on different lesions caused by chronic diseases including diabetes and bedsores were defined as Marjolin’s ulcers, and the incidence of Marjolin’s ulcers may gradually increase (21). Importantly several common malignancies are originated from Marjolin’s ulcers (8, 22), so in this study we investigate the relationship of Marjolin’s ulcers with tumor-related factor HER2 and p53.

HER2 is a member of the epidermal growth factor family and has been best characterised in breast cancer. Many reports have demonstrated that the expression of HER2 is existed in non-breast cancer (23). For example, expression of HER2 was found in salivary gland cancer, pancreatic cancer, laryngeal cancer, endometrial cancer, and intestinal cancer (12). Meanwhile, it has also been reported that there is only very little HER2 expression in basal cell carcinoma (24), whereas there is no expression of HER2 in laryngeal invasive squamous cell carcinoma (25). Consistent with this, our study confirmed that HER2 was not expressed in Marjolin’s ulcers samples.

p53 is a tumor suppressor gene and is associated with malignancy and prognosis of many tumors. For example, Mutation of p53 gene or interaction with oncogene products of DNA tumor virus can cause cancer (26). As reports shown that p53 is involved in cancers of the breast, liver, colon, lung, brain, esophagus, hemopoietic tissues and reticuloendothelial tissues (27). Importantly, in this study we found the expression of p53 in Marjolin’s ulcers (Fig. 2). Meanwhile, we showed that the positive rate of p53 in Marjolin’s ulcers is related to gender, ulcer size, pathological type of
tumor and degree of differentiation. Furthermore, we found that the degree of malignancy of Marjolin’s ulcer was proportional to the positive expression rate of p53, which indicated that p53 can be used as a marker of the malignant degree of Marjolin’s ulcer.

In summary, we demonstrated that Majorlin’s Ulcer samples did not express HER2 but expressed p53. Importantly, our results suggested that the positive expression rate of p53 in Majorlin’s Ulcer samples was associated with sex, course of disease, ulcer size, pathological type of ulcer caneration, and degree of tumor differentiation. Interestingly, we found that the positive expression rate of p53 was proportional to the malignancy level of Marjolin’s ulcer. This study suggests that P53 may be valuable for Marjolin’s ulcer clinical and prognostic judgment, while HER2 is not. Further research on this study will shed light on the mechanism of Majorlin’s Ulcer result in malignancies.

Declarations

Availability of data and materials

The data presented in this study can be shared in response to reasonable request to the corresponding author.

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Ethics declarations

Ethics approval and consent to participate

This study was approved by Wuhan Third Hospital Research Ethics Committee (No. KY-2018-041).

Consent to Participate /Consent for Publication: All patients in this study consented to their involvement.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Dongmei Jin obtained the diagnostic material from the archive of Pathological Department, carried out immunohistochemic tests and drafts the manuscript. Yan Xiao carried out pathological examination. Wen Ma carried out pathological examination, participated in the coordination of the study and contributed to the preparation of manuscript. Chen Xia participated in the experimental design, the coordination of the study, and the final preparation of manuscript. Zhigang Chu participated in experimental design. All authors read and approved the final manuscript.
Consent for publication

All authors of the manuscript have read and agreed to its content and are accountable for all aspects of the accuracy and integrity of the manuscript in accordance with ICMJE criteria.

That the article is original, has not already been published in a journal, and is not currently under consideration by another journal.

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Figures
Figure 1

The pathological types of Marjolin's Ulcer canceration (hematoxylin and eosin staining, magnification, ×100) A: Squamous cell carcinoma; B: Basal cell carcinomas; C: Sarcoma; D: Malignant melanoma.
Figure 2

Expression of HER2 in Marjolin's ulcer (hematoxylin staining, magnification, ×100) A: Negative expression of HER2 in squamous cell carcinoma; B: Negative expression of HER2 in Basal cell carcinomas; C: Negative expression of HER2 in sarcoma; D: Negative expression of HER2 in Malignant melanoma.
Figure 3

Expression of p53 in Marjolin’s ulcer (hematoxylin staining, magnification, ×100) A: Negative expression of p53 in squamous cell carcinoma; B: Expression of p53 in squamous cell carcinoma (2+); C: Expression of p53 in squamous cell carcinoma (3+); D: Expression of p53 in basal cell carcinomas (3+); E: Expression of p53 in malignant melanoma (3+); F: Negative expression of p53 in sarcoma.