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Original Article

MicroRNA26 increases sensitivity of cutaneous squamous cell carcinoma to dacarbazine

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Abstract: Cutaneum carcinoma threatens health and lives of patients. Dacarbazine is one of the most important therapeutics for cutaneum carcinoma. Current study was focused on molecular mechanism of sensibilization effect of microRNA26 on sensitivity of cutaneous squamous cell carcinoma to dacarbazine. Colon16 (cell line of cutaneous squamous cell carcinoma) was prepared for experiment. Para-carcinoma tissue and cutaneous squamous cell carcinoma were collected to compare expression of microRNA26. Synthetic microRNA26 and control microRNA were transfected into Colon16 with lipofection transfection, respectively. All Colon16 cells were treated with dacarbazine. The growth and apoptosis of Colon16 were examined by flow cytometry. RT-PCR was performed to examine expression level of microRNA26. Growth of Colon16 was significantly inhibited after dacarbazine treatment, while apoptosis and expression of microRNA26 increased. Compared with para-carcinoma tissue, cutaneous squamous cell carcinoma had a higher level of microRNA26. Overexpression of microRNA26 promoted dacarbazine-induced apoptosis of Colon16. Dacarbazine induced apoptosis of cutaneous squamous cell carcinoma via increasing expression of microRNA26, and microRNA26 increased sensitivity of cutaneous squamous cell carcinoma to dacarbazine.

Keywords: Dacarbazine, microRNA26, Colon16, apoptosis, cutaneous squamous cell carcinoma

Introduction

Cutaneous squamous cell carcinoma is a malignant skin disease caused by mutations and malignant hyperplasia of skin cells [1]. Cutaneum carcinoma threatens health and lives of patients [2]. Recent study indicated underlying mechanism of cutaneous squamous cell carcinoma included cell prosoplasia, increasing proliferation and inhibited apoptosis [3]. Cutaneous squamous cell carcinoma not only results in aberrant accumulation of cutaneous squamous cells ,but also lead to infiltration of cancer cells in multiple organs, including vagina, cervix, esophagus, lips and oral cavity [4, 5]. Surgery is the main treatment for early-stage cutaneous squamous cell carcinoma, while advanced cutaneous squamous cell carcinoma is treated by non-operation therapy, including chemotherapy, radiotherapy and comprehensive therapy. The therapeutic mechanism of chemotherapy is inhibiting cancer proliferation and promoting apoptosis via chemicals intervention [6].

Dacarbazine (DTIC) is an important chemotherapeutic widely used in clinical scenario, and has promising efficacy for malignant lymphoma, melanoma and soft tissue tumor [7]. Latest research showed combination of dacarbazine and other adjuvant therapies had an ideal efficacy for cutaneous squamous cell carcinoma, meanwhile, animal experiment demonstrated combination of dacarbazine and specific microRNA increased sensitivity of cutaneous squamous cells to dacarbazine [8]. However, molecular mechanism underlying dacarbazine efficacy for cutaneous squamous cell carcinoma was still unclear.

MicroRNA is a kind of small RNA fragments with a length of 17 bp to 27 bp. Although microRNA does not encode any proteins, it has many biology functions [9, 10], including cell viability, proliferation [11], apoptosis [12], cell cycle [13], autophagy [14], and intracellular signal transduction [15]. In the field of cutaneous squamous cell carcinoma, studies about microRNA are still scarce [16].

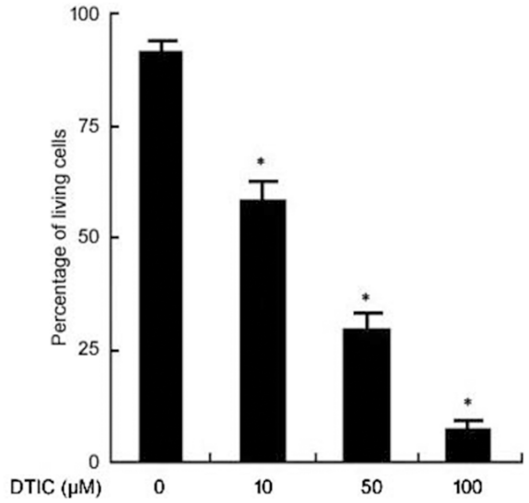

Figure 1. Growth of Colon16 was inhibited after dacarbazine treatment. DTIC represents dacarbazine. *P<0.05 versus control group (leftmost panel). All the experiments were performed three times yielding similar results and comprised of biological replicates. For all the figures, the mean ± standard deviation (s.d.) is represented unless otherwise noted.

Current study was aimed to explore not only regulatory mechanism underlying effect of dacarbazine on Colon16, but alsomolecular mechanism of how microRNA26 influenced sensitivity of Colon16 to dacarbazine so as to provide theory basis for clinical practice.

Materials and methods

Reagents and materials

Skin squamous carcinoma cells Colon16 were preserved in the laboratory. MTT used for detection of cell viability, as well as high glucose culture DMEM and fetal bovine serum were acquired from the Beijing Dingguo Biological Co., Ltd (Beijing, China). FITC-Annexin-V used for analysis of apoptosis and cell transfection reagents liposomes were provided by Beyotime biological technological research institute (Jiangsu, Haimen). Reverse transcription kit was purchased from Tiangen biological co., LTD. microRNA26 and control microRNA were synthesized by Jima biological co., LTD, sequences were shown as 5'ACAGTACGACGTGTAGCA3' and 5'ATGACGACAGACACGACT3'. Skin squamous cell carcinoma tissue and paracarcinorma tissue were provided by dermatology in our hospital and approved by our hospital ethics committee; the informed forms were signed by all skin squamous cell carcinoma patients.

Culture of skin squamous carcinoma cells Colon16

Skin squamous carcinoma cells were quickly transferred from liquid nitrogen to 37°C water bath for thawing, then centrifuging at 1000 rpm for 5 min, removing supernatant and resuspended in new DMEM and cultured at 37°C, 5% CO_2.

Transfection of skin squamous carcinoma cells Colon16

Skin squamous carcinoma cells cultured at 37°C, 5% CO_2 were reseeded in 96 wells plate (103/well) before transfection 24 h, when the density of cells reached at 80%, microRNA26 and control microRNA were diluted in culture by ratio of 1:100, mixing fully and stewing for 6 min. In the end, the mixture above was dropped in cell culture plate and shaken gently for fully reaction. The culture was changed after 24 h transfection.

MTT cell viability assay for skin squamous carcinoma cells Colon16

Preliminary test was performed based working concentration of dacarbazine [16], 10 μM, 50 μM and 100 μM dacarbazine were used respectively to treat skin squamous carcinoma cells Colon16 for 14 h. Three parallel traits were designed. MTT (5 mg/ml, 30 μl) reaction solution was added into three different skin squamous carcinoma cells Colon16, culturing at 37°C for 3 h. Washing cell above 3 times, and then DMSO was added into squamous carcinoma cells Colon16 to terminate reaction for 10 min. In the end, the optical density was detected for squamous carcinoma cells Colon16 at 492 nm in microplate reader.

Cell apoptosis assay

Skin squamous carcinoma cells Colon16 transfected with microRNA or treated with dacarbazine were selected, and cell apoptosis was detected by cytometer and caspase activity respectively. Cytometer were performed according to the introduction of kit, showed as following: adjusting the treated cell concentration at 105/ml, cell suspension was mixed with reac-

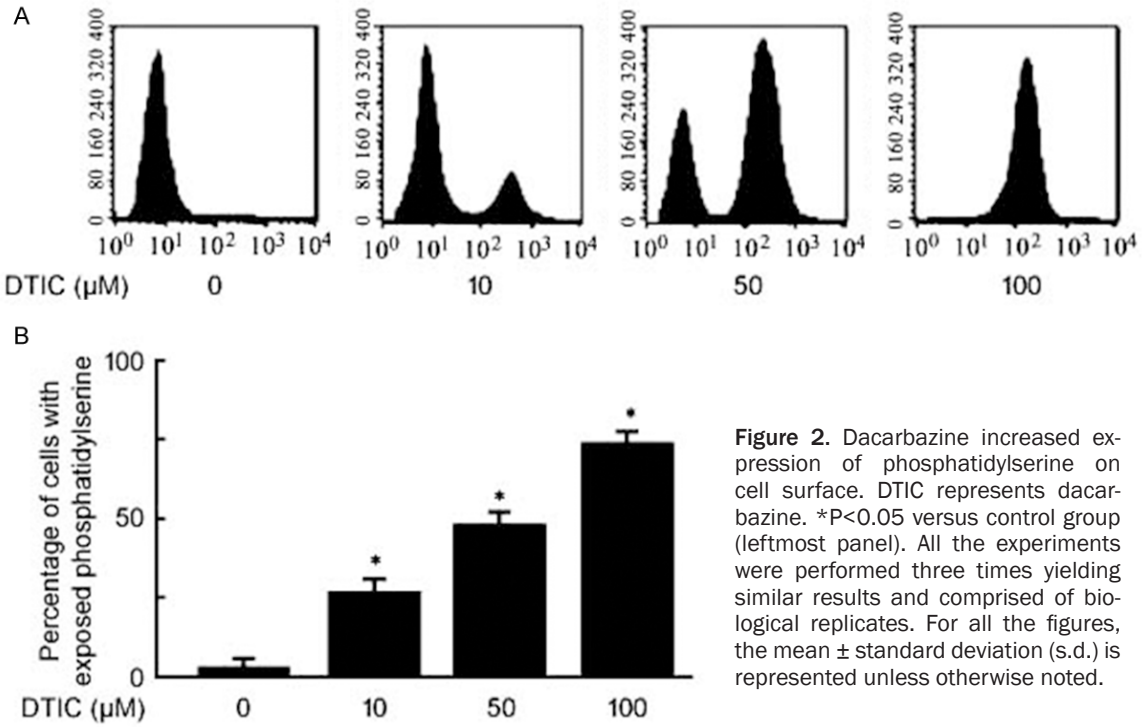

Figure 2. Dacarbazine increased expression of phosphatidylserine on cell surface. DTIC represents dacarbazine. *P<0.05 versus control group (leftmost panel). All the experiments were performed three times yielding similar results and comprised of biological replicates. For all the figures, the mean ± standard deviation (s.d.) is represented unless otherwise noted.

tion solution and FITC-Annexin V reagents by ratio of 250:50:1, reacting for 15 min at room temperature. Then expression of phosphatidylserine was detected in different skin squamous carcinoma cells Colon16, emission and absorb wavelength were 488 nm and 625 nm, respectively.

Activity assay were performed based the introduction of kit, specific steps shown as following: adjusting the treated cell concentration at 105/ml, cells transfected microRNA or treated with dacarbazine were lysed, then chromophoric substrate were added into the lysis, culturing 30 min at 37°C, in the end, absorbance value were assayed at 490 nm, which indicated the relative activity of caspase-3.

RT-PCR

Skin squamous carcinoma cells Colon16 transfected microRNA or treated with dacarbazine and Skin squamous carcinoma cells were collected, then total RNA was extracted by Trizol on the basis of the instruction of kit, then reversed transcript into cDNA, processed RT-PCR, microRNA26 and control microRNA primers were treated as primers, then the microRNA26 in skin squamous carcinoma cells Colon16 were detected.

Statistical method

All results were analyzed with SPSS12.0. All the experiments were performed three times yielding similar results and comprised of biological replicates. For all the figures, the mean ± standard deviation (s.d.) is represented unless otherwise noted. Unpaired Student's t test or ANOVA with Tukey's comparison were used for paired and group comparisons, respectively. Single factor variance was applied in the intergroup analysis. *P* value<0.05 was considered to be statistically significant.

Results

Dacarbazine inhibited growth of Colon16

Our finding on MTT experiment demonstrated dacarbazine inhibited growth of Colon16 in a dose-dependent manner. Compared with Colon16 untreated by dacarbazine, growth of all dacarbazine-treated Colon16 cell lines was significantly inhibited (**Figure 1**). What is more, dacarbazine concentration was negative with growth of Colon16 (P<0.05).

Dacarbazine induced apoptosis of Colon16

Experiment results of Colon16 showed that dacarbazine resulted in activation of caspase-3

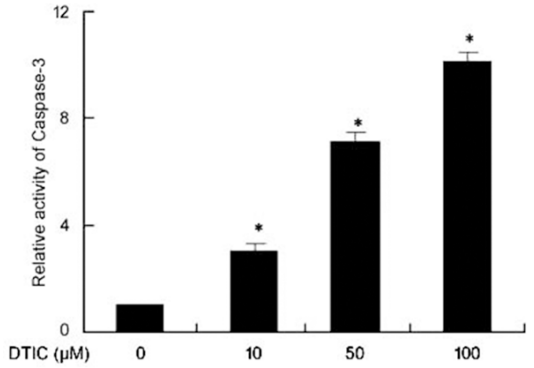

Figure 3. Dacarbazine resulted in activation of caspase-3. DTIC represents dacarbazine. *P<0.05 versus control group (leftmost panel). All the experiments were performed three times yielding similar results and comprised of biological replicates. For all the figures, the mean ± standard deviation (s.d.) is represented unless otherwise noted.

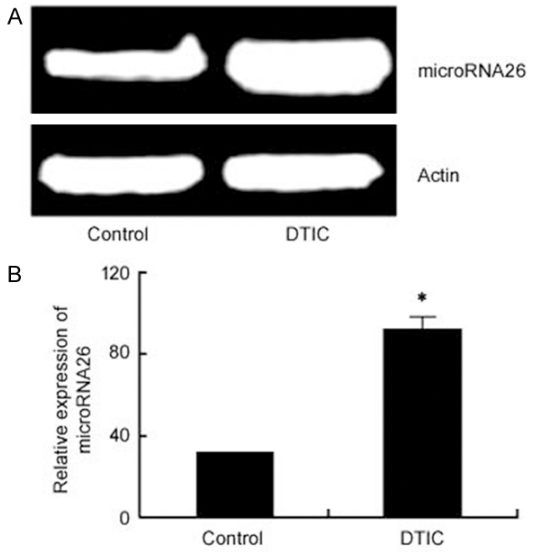

Figure 4. Expression level of microRNA26 was significantly increased in dacarbazine-treated Colon16. A. RT-PCR images. B. Analysis of microRNA26 expression. DTIC represents dacarbazine. The concentration of dacarbazine is 10 μM. *P<0.05 versus control group (left panel). All the experiments were performed three times yielding similar results and comprised of biological replicates. For all the figures, the mean ± standard deviation (s.d.) is represented unless otherwise noted.

and increased expression of phosphatidylserine on cell surface. Such effect of dacarbazine was also induced in a dose-dependent manner. Compared with Colon16 untreated by dacarbazine, apoptosis of all dacarbazine-treated Colon16 cell lines was significantly enhanced (P<0.05, **Figures 2** and **3**).

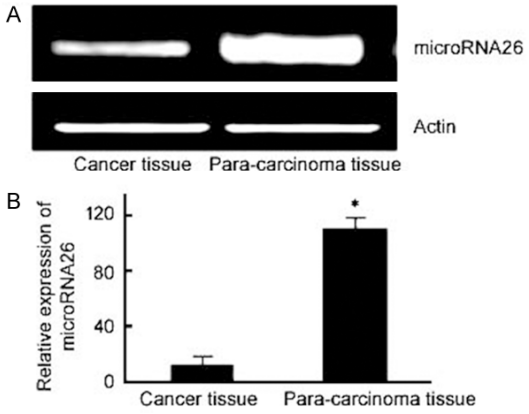

Figure 5. MicroRNA26 expression significantly decreased in cancer tissues from cutaneous squamous cell carcinoma patients. A. RT-PCR images. B. Analysis of microRNA26 expression. DTIC represents dacarbazine. *P<0.05 versus control group (left panel). All the experiments were performed three times yielding similar results and comprised of biological replicates. For all the figures, the mean ± standard deviation (s.d.) is represented unless otherwise noted.

Dacarbazine increased expression level of microRNA26 in Colon16

RT-PCR result showed, compared with control group, expression level of microRNA26 in Colon16 was significantly elevated after dacarbazine treatment (P<0.05, **Figure 4**). Such data indicated that microRNA26 increase might mediate dacarbazine-induced apoptosis of Colon16 (**Figure 4**).

MicroRNA26 expression significantly decreased in cancer tissues from cutaneous squamous cell carcinoma patients

Compared with para-carcinoma tissue, microRNA26 expression of cutaneous squamous cell carcinoma tissues significantly decreased, verified by RT-PCR examination (P<0.05, **Figure 5**). Such data suggested low expression level of microRNA26 was associated with disease progress of cutaneous squamous cell carcinoma.

MicroRNA26 transfection enhanced dacarbazine-induced apoptosis of Colon16

Our finding on transfection experiment showed both microRNA26 transfection and control microRNA transfection did not cause apoptosis of Colon16 at baseline. However, compared with control microRNA transfection, dacarba-

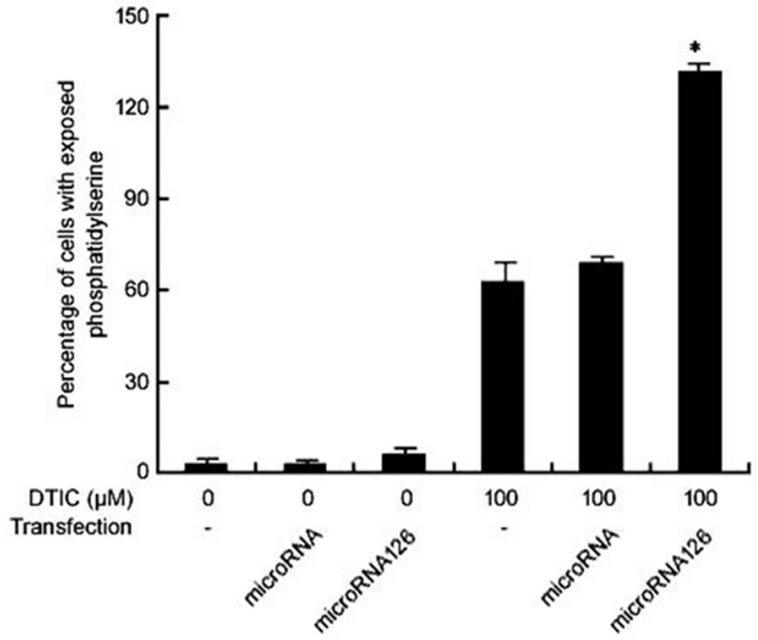

Figure 6. Overexpression of microRNA26 enhanced dacarbazine-induced apoptosis. DTIC represents dacarbazine. *P<0.05 versus control group (left-most panel). All the experiments were performed three times yielding similar results and comprised of biological replicates. For all the figures, the mean ± standard deviation (s.d.) is represented unless otherwise noted.

zine induced a higher apoptosis degree in microRNA26 transfected Colon16 (P<0.05, **Figure 6**), suggesting dacarbazine-induced apoptosis was associated with microRNA and overexpression of microRNA26 could enhance dacarbazine-induced apoptosis.

Discussion

Cutaneous squamous cell carcinoma is a world-wide disease, seriously impacting human health and lives [17]. Thus, to study this disease is of both theoretical significance and clinical value [18].

Dacarbazine plays a key role in clinical antineoplastic therapy [19]. However, it is unclear that how dacarbazine regulated cutaneous squamous cell carcinoma. Our study explored regulatory mechanism underlying effect of dacarbazine on cutaneous squamous cell carcinoma, verified by experiment conducted in cutaneous squamous cell carcinoma Colon16 cell line. We found that dacarbazine treatment inhibited growth of Colon16, induced apoptosis of Colon16. Moreover, such apoptosis inhibiting effect is dose-independent with dacarbazine, consistent with related studies in other types of cancers or other chemotherapeutics [20].

Three main findings were elucidated in our study. (1) Dacarbazine treatment suppressed growth of Colon16 and induced significant apoptosis of Colon16 with a marked increase of microRNA26 expression. (2) In patients with cutaneous squamous cell carcinoma, microRNA26 might be used as a predicting factor. (3) Overexpression of microRNA26 further induced apoptosis of cutaneous squamous cell carcinoma with clinical significance. These findings indicated that dacarbazine not only limited cancer growth, but also induced apoptosis of cutaneous squamous cell carcinoma via increasing expression of microRNA26, which finally increased sensitivity of cutaneous squamous cell carcinoma to dacarbazine.

Though similar with previous studies [21], our study stilled demonstrated disagreement. We observed that overexpression of microRNA26 alone cannot inhibit Colon16, while enhance inhibiting effect when combined with dacarbazine. One possibility is that every type of microRNA regulated different cancer with different effect [22], and some microRNA can induce opposite effect under different condition [23, 24]. Meanwhile, details of experimental condition differ among studies [25-27]. Further studies are warranted to answer these questions.

Our study had three limitations. (1) Only cell model was established to explore effect of dacarbazine, thus, animal experiment need to be conducted in cutaneous squamous cell carcinoma model [28]. (2) We found dacarbazine increased expression of microRNA26, but it was not yet to be seen how expression of microRNA26 changed at different stage of cancer. (3) Our study did not elucidated detailed mechanism underlying how microRNA26 increased sensitivity of cutaneous squamous cell carcinoma to dacarbazine.

In conclusion, microRNA26 enhanced sensitivity of cutaneous squamous cell carcinoma to

dacarbazine, and dacarbazine promoted cancer apoptosis via increasing expression of microRNA26.

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Disclosure of conflict of interest

None.

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