SKIN

BRIEF ARTICLE

Increased Invasiveness and Recurrence of Non-Melanoma Skin Cancer Associated with Previous Liquid Nitrogen Therapy: A Case Series Involving 16 Patients Facing Life-threatening Consequences Secondary to Dermatologic Mismanagement

David Roy, DO[1], Michael Coffin, BS[2]

[1] Pine Belt Dermatology and Skin Cancer Center, Petal, Mississippi, USA
[2] William Carey College of Osteopathic Medicine, Hattiesburg, Mississippi, USA

ABSTRACT

Introduction: Cryosurgery has long been used to treat skin lesions, often without histopathologic confirmation. This can result in the undertreatment of undiagnosed malignancies. We describe 16 patients with non-melanoma skin cancers (NMSCs) exhibiting high-risk features following repeated, and at times inappropriate, cryosurgery over months to years.
Case Series: Among the 16 cases, 13 were invasive squamous cell carcinomas (SCC) and 3 were invasive basal cell carcinomas (BCC), all ultimately treated with Mohs micrographic surgery (MMS). Of the SCCs, 6 (46.2%) showed perineural invasion (PNI) on frozen section. PNI was also seen in 1 of the 3 BCC cases. Four carcinomas (3 SCCs and 1 BCC) were located on the forehead.
Discussion: Emerging evidence suggests that chronic injury may promote carcinogenesis via stem cell lineage infidelity driven by sustained stress signals in the skin's microenvironment. While transient infidelity aids wound healing, persistent dysregulation is a hallmark of cancer. Repeated cryosurgery may create such a pro-tumorigenic environment, leading to aggressive transformation of precursor lesions.
Conclusion: These cases highlight a concerning pattern of aggressive NMSC developing after repeated cryosurgery for lesions initially presumed benign. Current guidelines designate tumors arising in chronically inflamed or previously treated sites as high-risk. We propose that repeated cryosurgical destruction should similarly qualify as a high-risk factor. Limitations include the absence of histologic assessment prior to cryotherapy, emphasizing the need for biopsy in persistent or recurrent lesions.

INTRODUCTION

Cutaneous squamous cell carcinoma (CSCC) is the second most common type of skin cancer, causing an average of 700,000 new cases each year.[1] The death rate has been estimated to be 1.5–2.1%, and CSCC is responsible for 4,000–9,000 mortalities annually.[2] Basal cell carcinoma (BCC), the most common type of skin cancer, accounts for approximately 3.6 million new cases each year, but it has a much lower mortality rate, with deaths being extremely rare.[1] Among

patients with CSCC, 2.5–14% are concurrently diagnosed with perineural invasion (PNI), which confers a more aggressive subtype with poorer prognosis.[3-5] PNI is a mechanism by which neoplastic growth and spread occur in and around nerves, taking advantage of areas of minimal resistance. Tumors exhibiting PNI have elevated risk of distant metastases, and elevated rates of recurrence, morbidity, and mortality. Squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) are among the most common cutaneous tumors known to show these phenomena.[2-7] Poor tumor differentiation, recurrence, depth of extension, and location in the head and neck region are known risk factors for PNI.[6,7] Additionally, destructive methods such as cryosurgery have been implicated in PNI development.[8]

In the current dermatologic literature, very little has been written addressing the potential deleterious effects resulting from repeated destruction of malignant lesions with cryosurgery. A recent report comparing incidental PNI (IPNI) and clinical PNI (CPNI) has determined that patients who show clinical symptoms and/or radiographic evidence of PNI (CPNI) are at greater risk of recurrence and death than those for whom PNI is detected incidentally in histologic examination during Mohs surgery (IPNI).[9] Furthermore, in the absence of such risk factors, CSCCs that invade smaller caliber nerves (less than 0.1 mm in diameter) have been proposed to have better outcomes than those displaying PNI of large-caliber nerves.[7] The local tissue destruction occurring as a result of use of cryotherapy for removal of non-melanoma skin cancer might potentially decrease resistance to tumor growth and spread.[10]

Cryosurgery was first introduced as an effective method of treatment for non-melanoma skin cancers in 1907.[11] Because of limitations in the delivery of liquid air, cryosurgery was initially abandoned; however, it was later reintroduced in the treatment of non-melanoma skin cancers, and the open-spray technique was found to be a safe, low-cost, effective method with a high cure rate.[12,13] Liquid nitrogen is now accepted as the most effective and widely used cryogen to treat common skin lesions.[14] This low-cost, easily performed technique has an additional advantage of not requiring patients to adhere to long-term application of topical medications, such as in the treatment of actinic keratosis, which have a 75–99% efficacy when used properly.[15] Disadvantages of cryosurgery include blistering, crusting, hypopigmentation, and general discomfort from direct application of liquid nitrogen.[16] Herein, we describe 16 cases: 8 diagnosed histologically as moderately differentiated SCC, 4 diagnosed as poorly differentiated SCC, 1 diagnosed as a well-differentiated SCC, and 3 diagnosed as infiltrative BCC. Of the 16 cases, 7 exhibited PNI (43.8%), and all tumors had been repeatedly subjected to liquid nitrogen therapy over the course of months to years.

CASE SERIES

Patient 1

A 63-year-old white man presented to our dermatology clinic with a chief complaint of a skin lesion above his left eye. He reported prior therapy at a different local “dermatology” clinic, where he was treated with cryotherapy with liquid nitrogen on 4 separate occasions over the course of 12 months. That clinic, owned and operated by a board-certified pediatrician, did not perform a biopsy during that period. Over time, the lesion persisted; because of the lack of response to liquid nitrogen therapy, the

patient sought a second opinion at our dermatology clinic.

Physical examination revealed a 4 cm erythematous plaque with a central depressed region and associated telangiectasia (**Figure 1A**). Because of concern for non-melanoma cutaneous malignancy, a shave biopsy was obtained and sent for histologic analysis, from which the patient received a diagnosis of moderately differentiated SCC. Furthermore, evidence of small-caliber perineural invasion was observed (**Figure 1B**). The patient was scheduled for MMS, given the size, location, and histopathologic findings associated with the diagnosis. Clear margins were eventually obtained after 3 stages of MMS, which left a 5 cm × 4 cm defect (**Figure 1C**).

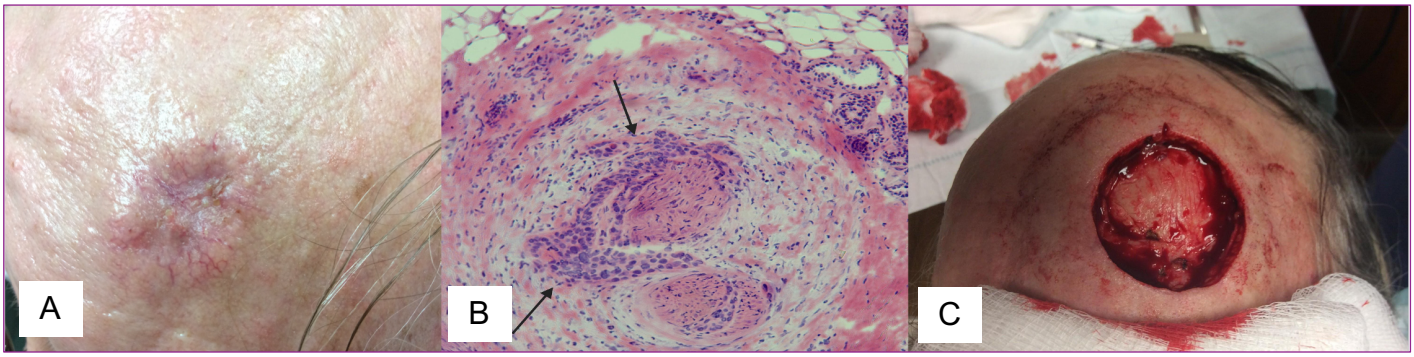

Figure 1. (A) Left. Gross inspection of the left supraorbital lesion exposed to 4 rounds of liquid nitrogen cryotherapy over the course of 1 year, without biopsy. (B) Middle. Perineural invasion of a small-caliber nerve sheath (arrows) observed at the time of histologic diagnosis and during Mohs micrographic surgery (MMS). (C) Perioperative defective after confirmation of clear margins during MMS.

Patient 2

A 62-year-old white man presented to our dermatology clinic with a chief complaint of a skin lesion in front of his right ear. He reported having sought care at the same dermatology office as patient 1. He reported that he was administered cryotherapy on 7 occasions over the course of 24 months, and that the lesion was never biopsied. Because of failed therapy and continued lesion growth, the patient sought a second opinion.

Gross inspection of the patient's head revealed a 7 cm erythematous plaque with crusting and raised borders that raised concern for a cutaneous neoplasm (**Figure 2A**). Histopathologic examination of a tissue biopsy obtained at the time of presentation was highly suggestive of infiltrative BCC with perineural involvement (**Figure 2B**). The patient was scheduled for definitive therapy with MMS, which necessitated a large defect to obtain positive margins, as depicted in **Figure 2C**.

We observed a clear correlation between repeated use of liquid nitrogen cryotherapy and heightened aggressiveness of malignancies (**Table 1**). Of the 16 cases, 7 exhibited PNI (43.8%). Among patients with a diagnosis of SCC, 6 exhibited PNI (46.2%), including the 1 case of well-differentiated subtype (100%), 2 of the 4 cases of poorly differentiated subtype (50%), and 3 of the 8 cases of moderately differentiated subtype (37.5%). Additionally, 1 of the 3 cases of infiltrative BCC exhibited PNI (33%). Moreover, carcinomas located on the forehead (3 SCC and 1 BCC) were often associated with PNI (75% of cases).

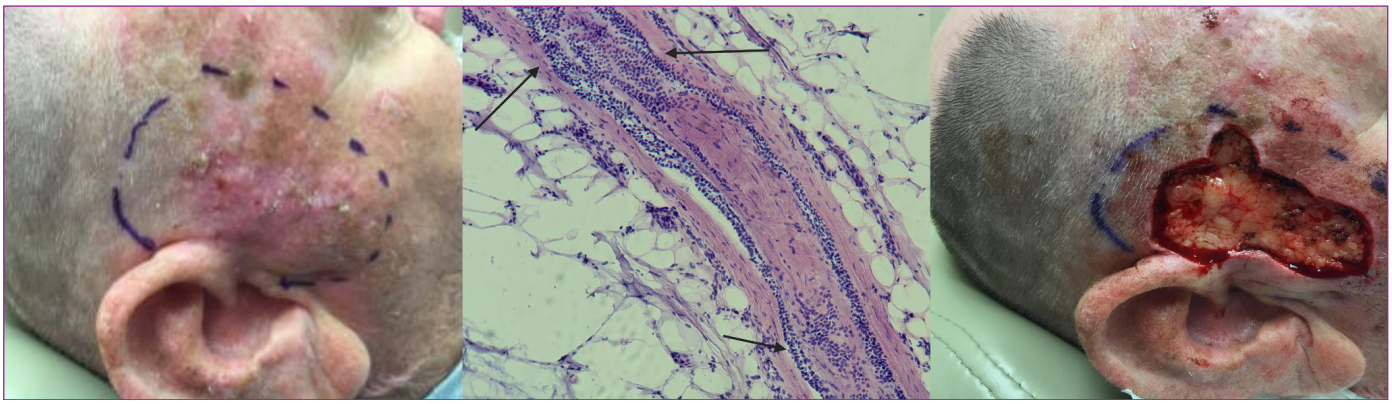

Figure 2. (A) Left. Gross appearance of the right preauricular lesion subjected to 7 prior rounds of cryotherapy over 2 years. (B) Middle. Evidence of basal keratinocytes invading the perineural sheath of a local small-caliber cutaneous nerve. (C) Right. Surgical defect after confirmation of tumor excision during MMS.

DISCUSSION

PNI is known to occur in a wide variety of both cutaneous and non-cutaneous malignancies. Cutaneous SCC and BCC tend to spread through PNI.[1] In a review of 520 patients with cutaneous SCC of the face, Goepfert et al. have reported a diminished rate of survival in patients with PNI.[2] In a prospective study of 1263 patients treated with MMS between 1993 and 2002, Leibovitch et al. found that lesser degrees of differentiation on histology are associated with prior recurrence.[3] The same study has also indicated that destructive modalities such as cryotherapy, when used to treat recurrent skin malignancies, may potentially induce architectural changes in the surrounding tissue and subsequently lead to difficulties in margin control for later excisions. The second part of that study, involving a 5-year follow-up of 70 patients diagnosed with PNI, showed that PNI was associated with more aggressive tumors. PNI was also associated with greater numbers of prior surgical excisions and cryotherapy treatments.

Maintaining a high index of suspicion is important in the determination of certain PNI-specific characteristics, so that the most effective treatment modality can be used. For instance, distinguishing between clinical perineural invasion (CPNI) and incidental perineural invasion (IPNI) is key to understanding the degree to which a certain tumor exhibits PNI. Most patients (60–70%) present with IPNI, and have no prior radiological or clinical evidence of any such symptoms pertaining to PNI.[22] In contrast, a minority of patients (30–40%) present with CPNI, which describes tumors with clinical findings secondary to the presence of PNI, such as numbness, tingling, paralysis, and formication.[23] In a retrospective cohort study, Karia et. al. have reported a significantly greater risk of local recurrence and death from CSCC in patients who present with CPNI.[24]

Moreover, the size of the involved nerves is another important prognostic factor in PNI. Carter et. al. have determined that the involvement of larger named nerves in tumor growth and spread correlates with poorer outcomes in patients with PNI than in patients whose PNI is limited to smaller caliber nerves less than 0.1 mm in diameter.[7] Therefore, physicians must assess the degree of nerve involvement according to size, as well as the presence/absence of any clinical symptoms associated with nerve involvement by the

Table 1. Patient information, showing an association between liquid nitrogen exposure and aggressive neoplastic behaviors in 16 patients treated by the same board-certified pediatrician advertising dermatology services

Patient	Location	Prior Exposure to Liquid Nitrogen	Histologic Diagnosis	Evidence of PNI
Patient 1, 63-year-old white man	Left supraorbital	4 times in 12 months	Moderately differentiated SCC	Yes
Patient 2, 64-year-old white man	Right pre-auricular	7 times in 24 months	Infiltrative BCC	Yes
Patient 3, 73-year-old white man	Right forehead	5 times in 17 months	Moderately differentiated SCC	No
Patient 4, 52-year-old white man	Right lower lip	2 times in 3 months	Moderately differentiated SCC	Yes
Patient 5, 69-year-old white man	Right pre-auricular	5 times in 24 months	Poorly differentiated SCC	Yes
Patient 6, 79-year-old white man	Nasal apex	4 times in 14 months	Moderately differentiated SCC	No
Patient 7, 77-year-old white man	Left ear, helix	6 times in 16 months	Infiltrative BCC	No
Patient 8, 81-year-old white woman	Left forehead/temporal area	≥10 times in 4 years	Infiltrative BCC	Yes
Patient 9, 77-year-old white man	Scalp	3 times in 12 months	Moderately differentiated SCC	Yes
Patient 10, 83-year-old white man	Scalp	8 times in 24 months	Poorly differentiated SCC	No
Patient 11, 71-year-old white woman	Right temporal	≥8 times in 36 months	Poorly differentiated SCC	No
Patient 12, 61-year-old white man	Left inferior nare and nasal septum	4 times in 15 months	Moderately differentiated SCC	No
Patient 13, 81-year-old white man	Scalp	7 times in 24 months	Moderately differentiated SCC	No
Patient 14, 60-year-old white man	Left supraorbital	4 times in 12 months	Well differentiated SCC	Yes
Patient 15, 59-year-old white man	Lower lip	3 times in 15 months	Moderately differentiated SCC	No
Patient 16, 72-year-old white man	Left ear	≥10 times in 27 months	Poorly differentiated SCC	Yes

tumor, to decide on the aggressiveness of the treatment approach. Patients with CPNI and/or histologic evidence of large-caliber nerve involvement are candidates for aggressive treatment and close surveillance for recurrence.

CSCC is a type of skin cancer known for its aggressive behavior and recurrence risks, partly because of perineural invasion (PNI), thus extending the clinical implications of PNI.[25] Recent research has highlighted the importance of stem cell lineage infidelity in CSCC progression, wherein cancer stem cells' deviation from their original lineage pathways leads to tumor heterogeneity and increased malignancy.[26] Guan et al have emphasized the role of ETS2 in inducing stem cell lineage infidelity in skin epithelia, which resemble activated stem cells during wound repair and tumorigenesis.[26] Moreover, SOX2 has been shown to be a biomarker involved in regulating cutaneous cancer stem cell function, thus suggesting a continuum between tumor initiation and progression in skin SCCs.[27]

Understanding the molecular mechanisms underlying stem cell lineage infidelity in CSCC is crucial for prognosis and treatment strategies. The reciprocal communication between the epidermis and dermis, along with the cellular heterogeneity and plasticity within epidermal stem cells and their niches, substantially influences skin development, repair, and cancer progression [28,29]. Additionally, the expression of PITX1 controls transcriptional circuits governing self-renewal and differentiation in SCC, thus underscoring the importance of lineage relationships between normal and malignant progenitor cells.[30]

The implications of stem cell lineage infidelity in CSCC research extend to potential therapeutic targets. The skin epithelium provides a model for determining lineage relationships between normal and malignant progenitor cells, which may offer insights into the development of targeted therapies.[30] Furthermore, the presence of pre-malignant mutations in sun-exposed skin suggests positive clonal selection even under normal physiological conditions, thus emphasizing the need for targeted interventions to address early molecular changes associated with CSCC.[31]

Another factor that should be considered in CSCC is the underlying genetic components potentially driving tumorigenesis. UVB light is well known to contribute to the formation of CSCC, and is believed to induce mutations in transcription factors such as p53, nuclear factor-kappa B (NF-κB), and activator protein-1 (AP-1).[32]Mutations in these transcription factors lead to hallmarks of tumor formation, such as resistance to apoptotic signals, limitless replicative potential, and metastatic capacity. UVB-induced mutations in the p.Ser24Phe region of the *KNSTRN* gene might potentially enhance *in vivo* tumorigenesis.[33] Micro-RNAs (miRNAs) have also been implicated as drivers of CSCC development. Recent reports focusing on miRNAs, specifically, miR-365, have yielded promising insights into the genetic pathway underlying CSCC tumorigenesis.[34] Genetic analysis supports that both Nuclear Factor I/B (NFIB) and BAX are targeted by miR-365, thus facilitating *in vivo* growth and development of apoptosis-resistant CSCC.[35-36] Whether the application of liquid nitrogen might hasten the development of the aforementioned mutations and subsequently promote the growth and spread of cutaneous non-melanoma skin cancer remains to be determined.

CONCLUSION

Our case series prompts a critical question of whether recurrent and/or inappropriate use of cryosurgical destruction might lead to aggressive progression of relatively stable tumors. Our patients' lesions had been assumed to be actinic keratoses or superficial non-melanoma skin cancers by the treating physicians. Currently, accepted practice suggests that tumors that are recurrent or located at sites of chronic inflammatory processes should be considered high risk. We believe that repeated and prolonged courses of cryosurgical destruction might be considered qualifying events for these two guidelines. This rudimentary report has several limitations, including a lack of histologic evaluation before cryosurgical treatment.

Conflict of Interest Disclosures: None

Funding: None

Corresponding Author:
Name
Address
Phone:
Email: hyperlink blue

References:

1. Rogers, HW, Weinstock, MA, Feldman, SR, Coldiron, BM. *Incidence estimate of nonmelanoma skin cancer (keratinocyte carcinomas) in the US population, 2012*. JAMA Dermatol 2015;151(10):1081-1086
2. Karia, PS, Han, J, Schmults, CD. *Cutaneous squamous cell carcinoma: estimated incidence of disease, nodal metastasis, and deaths from disease in the United States, 2012*. J Am Acad Dermatol 2013;68(6):957-966
3. Ballantyne, AJ. *Perineural invasion by SCC.* J Dermatol Surg Oncol. 1984; 10(7): 502-504
4. Immerman, SC, Scanlon, EF, Christ, M, Knox, KL. *Recurrent squamous cell carcinoma of the skin*. Cancer;51(8):1537-1540
5. Schmults, CD, Karia, PS, Carter, JB, Han, J, Qureshi, AA. *Factors predictive of recurrence and death from cutaneous squamous cell carcinoma: a 10 year, single-institution cohort study.* JAMA Dermatol 2013;149(5): 541-547
6. Rowe, DE, Carroll, RJ, Day, CL Jr. *Prognostic factors for local recurrence, metastasis, and survival rates in squamous cell carcinoma of the skin, ear, and lip.* J Acad Dermatol Surg 2006;32(11): 1309-1321
7. Carter, JB, Johnson, MM, Chua, TL, Karia, PS, Schmults, CD. *Outcomes of primary cutaneous squamous cell carcinoma with perineural invasion*. JAMA Dermatol 2013;149(1):35-41
8. Leibovitch, I, Huilgol, S, Selva, D, Hill, D, Richards, S, Paver, R. *Cutaneous squamous cell carcinoma treated with Mohs micrographic surgery in Australia II. Perineural Invasion*. J Am Acad Dermatol 2005;53:261-6
9. Karia, PS, Morgan, FC, Ruiz, ES, Schmults, CD. *Clinical and incidental perineural invasion of cutaneous squamous cell carcinoma*. JAMA Dermatol, 2017;153(8):781-788
10. Farasat, S, Yu, S, Neel, VA, et al. *A new American Joint Committee on Cancer staging system for cutaneous squamous cell carcinoma: Creation and rationale for inclusion of tumor (T) characteristics*. J Am Acad Dermatol 2011;64(6):1051-1059
11. Whitehouse HH. *Liquid air in dermatology: its indications and limitations*. J Am Med Assoc 1907; 49: 371-7.
12. Zacarian SA. *Cryosurgery for Skin Cancer and Cryogenic Techniques in Dermatology*. Springfield IL: Charles C Thomas, 1969; 11-21.
13. Holt, PJA. *Cryotherapy for skin cancer: results over a 5-year period using liquid nitrogen spray cryosurgery.* British Journal of Dermatology (198) 119, 231-240.
14. Andrews, MD. *Cryosurgery for Common Skin Conditions. American Family Physician* 2004; 69 (10), 2365-2372
15. Berlin JM. *Current and emerging treatment strategies for the treatment of actinic keratoses*. Clin Cosmet Investig Dermatol. 2010;3:119-26.
16. Feasel, AM, Brown, TJ, Bogle, MA, Tschen, JA, Nelson, BR. *Perieneural invasion of cutaneous malignancies*. Dermatol Surg 2001;27:531-42
17. Feasel AM, Brown TJ, Bogle MA et al. *Perineural invasion of cutaneous*

malignancies. Dermatol. Surg 2001;27: 531–42.
18. Brantsch, KD, Meisner, C, Schonfisch, SR, et al. *Analysis of risk factors determining prognosis of cutaneous squamous-cell carcinoma: a prospective study*. Lancet Oncol, 2008;9(8):713-720
19. Goepfert, H, Dichtel, WJ, Medina, JE, Lindberg, RD, Luna, MD. *Perninueral invasion in squamous cell skin carcinoma of the head and neck.* Am J Surgery, 1984; 148: 542-7
20. Leibovitch, I, Huilgol, S, Selva, D, Hill, D, Richards, S, Paver, R. *Cutaneous squamous cell carcinoma treated with Mohs micrographic surgery in Australia I. Experience over 10 years*. Dermatol Surg 2005; 253-60
21. Leibovitch, I, Huilgol, S, Selva, D, Hill, D, Richards, S, Paver, R. *Cutaneous squamous cell carcinoma treated with Mohs micrographic surgery in Australia II. Perineural Invasion*. J Am Acad Dermatol 2005;53:261-6
22. Cottel, WI. *Perineural Invasion by Squamous Cell Carcinoma*. J Dermatol Surg Oncol 1982;8(7): 589-600
23. Mendenhall, WM, Parsons, JT, Mendenhall, NP, et. al. *Carcinoma of the skin of the head and neck with perineural invasion*. Head Neck. 1989;11(4):301-308
24. Karia, PS, Morgan, FC, Ruiz, ES, Schmults, CD. *Clinical and incidental perineural invasion of cutaneous squamous cell carcinoma*. JAMA Dermatol, 2017;153(8):781-788
25. Guan Y., Wang G., Fails D., Nagarajan P., & Ge Y.. Unraveling cancer lineage drivers in squamous cell carcinomas. Pharmacology & Therapeutics 2020;206:107448.
26. Guan Y., Yang Y., Nagarajan P., & Ge Y.. Transcriptional and signaling regulation of skin epithelial stem cells in homeostasis, wounds and cancer. Experimental Dermatology 2020;30(4):529-545.
27. Sánchez-Danés A. and Blanpain C.. Deciphering the cells of origin of squamous cell carcinomas. Nature Reviews Cancer 2018;18(9):549-561.
28. Rognoni E. and Watt F.. Skin cell heterogeneity in development, wound healing, and cancer. Trends in Cell Biology 2018;28(9):709-722.
29. Lichtenberger B. and Kasper M.. Cellular heterogeneity and microenvironmental control of skin cancer. Journal of Internal Medicine 2020;289(5):614-628.
30. Sastre-Perona A., Hoang-Phou S., Leitner M., Okuniewska M., Meehan S., & Schöber M.. De novo pitx1 expression controls bi-stable transcriptional circuits to govern self-renewal and differentiation in squamous cell carcinoma. Cell Stem Cell 2019;24(3):390-404.e8.
31. Ge Y. and Fuchs E.. Stretching the limits: from homeostasis to stem cell plasticity in wound healing and cancer. Nature Reviews Genetics 2018;19(5):311-325.
32. Cooper SJ, Bowden GT. Ultraviolet B regulation of transcription factor families: roles of nuclear factor-kappa B (NF-kappaB) and activator protein-1 (AP-1) in UVB-induced skin carcinogenesis. Curr Cancer Drug Targets. 2007 Jun; 7(4):325-34.
33. Lee, CS, Bhaduri, A, Mah, A, et al. Recurrent point mutations in the kinetochore gene KNSTRN in cutaneous squamous cell carcinoma. Nat Genet 2014;46(10): 1060-1062
34. Zhou, M, Liu, W, Ma, S, et al. A novel onco-miR-365 induces cutaneous squamous cell carcinoma. Carcinogenesis 2013; 34(7): 1653-1659
35. Zhou, L, Gao, R, Wang, Y, Zhou, M, Ding, Z. Loss of BAX by miR-365 promotes cutaneous squamous cell carcinoma progression by suppressing apoptosis. Int J Mol Sci 2017;18(6): 1157-1160
36. Zhou, M, Zhou, L, Zheng, L, et al. miR-365 promotes cutaneous squamous cell carcinoma through targeting Nuclear Factor I/B (NFIB). PLoS One 2014;9(6): e100620
37. Ge Y, et al. Stem Cell Lineage Infidelity Drives Wound Repair and Cancer. Cell. 2017 May 4;169(4):636-650.e14.