**Supplementary information**

***Supplementary Materials and Methods***

***Relative quantification of gene expression by real-time PCR***

Samples for molecular analyses could be collected in 24 RDEB-SCCs including n=6 for low-risk primary SCC, n=3 for high-risk primary SCC, n=7 for low-risk recurrent SCC, and n=8 for high-risk recurrent SCC, as well as n=8 for non-lesional skin and n=7 for peri-tumoral skin in our study.

Total RNA was extracted from 5mm skin biopsies using RNeasy Fibrous Tissue Mini Kit (Qiagen, Redwood, CA). Reverse transcription was carried out with 500 ng of total RNA using Super Script IV Reverse transcription kit (Life Technologies, Carlsbad, CA). cDNA was amplified using Mesa Green qPCR kits for SYBR Assay (Eurogentec, Liège, Belgium) and Applied Biosystems 7300 Real-Time PCR System (Life Technologies). Primers for each target gene are listed in **Supplementary Table S4**. Relative gene expression was normalized to a housekeeping gene (PGK) and calculated using the 2ˆ-ΔΔCt method.

***Supplementary tables***

**Supplementary table S1.** Supplementary RDEB patient and SCC tumor sample details

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Patient | Sex, Age at the initial diagnosis | Survival after the initial  diagnosis (months) | Number of SCCs | Cause of death | Location, type of SCC | Differentiation classification | **group** | **FFPE** | **RNA** |
| 1 | M, 43 | 65 | 4 | alive | Hands | Well | **Low-risk pSCC** | **√** |  |
| Primary and recurrence | Well | **Low-risk rSCC** |  | **√** |
|  | Well | **Low-risk rSCC** | **√** |  |
| 2 | F, 27 | 109 | 4 | alive | Foot, Lower leg | Well\* | **High-risk rSCC** | **√** | **√** |
| Recurrence |
| 3 | F, 25 | 129 | 3 | alive | Lower leg | Well | **Low-risk rSCC** | **√** | **√** |
| Recurrence |
| 4 | F, 18 | 168 | 17 | gastrointestinal amyloidosis | Foot, Lower leg, Uper leg | Well | **Low-risk rSCC** | **√** |  |
| Recurrence | Well | **Low-risk rSCC** | **√** |  |
|  | Well | **Low-risk rSCC** | **√** |  |
|  | Well | **Low-risk rSCC** |  | **√** |
| 5 | F, 34 | 253 | 8 | alive | Neck | Well | **Low-risk rSCC** | **√** |  |
| Recurrence | Well | **High-risk rSCC** | **√** |  |
| 6 | M, 28 | 194 | 13 | sepsis | Lower leg | Well | **Low-risk rSCC** | **√** |  |
| Recurrence |
| 7 | M, 31 | 14 | 3 | metastasis | Hands | Poor | **High-risk pSCC** | **√** | **√** |
| Primary | Moderate | **Low-risk pSCC** | **√** |  |
| 8 | M, 34 | 175 | 11 | alive | Back, Lower leg | Well | **Low-risk rSCC** | **√** | **√** |
| Rrecurrence | Well | **Low-risk rSCC** |  | **√** |
| 9 | M, 33 | 229 | 8 | alive | Low leg | Well | **Low-risk rSCC** | **√** | **√** |
| Recurrence |
| 10 | F, 27 | 14 | 6 | metastasis | Back | Poor | **High-risk pSCC** | **√** |  |
| Primary, recurrence and | Moderate | **High-risk rSCC** |  | **√** |
| metastasis | Well | **High-risk rSCC** | **√** | **√** |
|  | Poor | **High-risk rSCC** | **√** | **√** |
| 11 | F, 25 | 16 | 3 | extensive SCC | Low leg | Well | **High-risk pSCC** | **√** |  |
| Primary and recurrence | Poor | **High-risk rSCC** | **√** |  |
| 12 | F, 21 | 60 | 2 | alive | Foot, Lower leg | Well | **Low-risk pSCC** | **√** | **√** |
| Primary and recurrence | Moderate | **Low-risk rSCC** | **√** |  |
| 13 | F, 39 | 56 | 2 | metastasis | Hands | Well | **Low-risk pSCC** | **√** | **√** |
| Primary and recurrence | Moderate | **High-risk rSCC** |  | **√** |
| 14 | F, 33 | 56 | 1 | alive | Neck | Well | **Low-risk pSCC** | **√** | **√** |
| Primary |
| 15 | F, 27 | 5 | 2 | sepsis | Upper leg | Well | **Low-risk pSCC** | **√** | **√** |
| Primary |
| 16 | F, 48 | 137 | 2 | alive | Lower leg | Well | **Low-risk rSCC** | **√** | **√** |
| Recurrence |
| 17 | F, 22 | 26 | 3 | sepsis | Lower leg | Poor | **High-risk pSCC** | **√** | **√** |
| Primary and recurrence | Moderate | **High-risk rSCC** | **√** | **√** |
| 18 | M, 20 | 13 | 5 | extensive SCC | Back | Poor | **High-risk pSCC** | **√** | **√** |
| Primary | Moderate | **High-risk rSCC** | **√** | **√** |
|  | Poor | **High-risk rSCC** | **√** | **√** |
| 19 | F, 30 | 28 | 2 | alive | Hand | Well | **Low-risk pSCC** |  | **√** |
| Primary |
| 20 | F, 31 | 15 | 4 | alive | Upper leg, Foot | Well | **Low-risk rSCC** | **√** |  |
| Recurrence | Well | **Low-risk rSCC** | **√** |  |
| SCCs cutaneous squamous cell carcinomas, FFPE formalin fixed and paraffin embedded, F Female, M Male, LG-pSCC low risk primary SCC, HG-pSCC high-risk primary SCC, LG-rSCC low-risk recurrent SCC, HG-rSCC high-risk recurrent SCC. | | | | | | | | | |  |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Supplementary table S2.** Aggressiveness parameters in high-risk SCC groups | | | | | | |
|  | |  | HR-pSCC (n=5)  n (%) | | | HR-rSCC (*n=10*)  n (%) |
| Local recurrence or metastasis at 3 months | | | | 4 (88.0) | 6 (60.0) | |
| Massive local expansion – limb amputation recommendation | | | | 1 (20.0) | 5 (50.0) | |
| Histopathological criteria: | Invasion depth ≥ 6 mm | | | 2 (40.0) | 5 (50.0) | |
|  | Lympho-vascular space invasion | | | 0 (0.0) | 1 (10.0) | |
|  | Perineural invasion | | | 0 (0.0) | 2 (20.0) | |
|  | Poorly differentiated | | | 4 (88.0) | 3 (30.0) | |

HR-pSCC high-risk primary SCC, HR-rSCC high-risk recurrent SCC

**Supplementary table S3.** Listing of antibodies

|  |  |  |
| --- | --- | --- |
| **Antibody** | **Source** | **Reference** |
| KI67 | Leica | RTU-Ki67-MM1 |
| CD20 | Abcam | ab64088 |
| CD3 | Abcam | ab5690 |
| CD4 | Dako | M7310 |
| CD8 | Abcam | ab4055 |
| Myeloperoxidase | R&D System | MAB3174 |
| Tryptase | Abcam | ab2378 |
| Citrullinated histone H3 (cit R2+R8+R17) | Abcam | ab5103 |

**Supplementary table S4.** Listing of primers used for RT-quantitative PCR

|  |  |  |
| --- | --- | --- |
| **Gene** | **Forward Primer** | **Reverse Primer** |
| *CDH1* | CGGACGATGATGTGAACACC | TTGCTGTTGTGCTTAACCCC |
| *CSF2* | AGCCGACCTGCCTACAGAC | AAGGGGATGACAAGCAGAAA |
| *CSF3* | CCACGAATTTGCTGGGGAAT | CTTTCACACACAGGCCTGAC |
| *CXCL8* | ACTCCTTGGCAAAACTGCAC | AAACCAAGGCACAGTGGAAC |
| *HMGB1* | TAGGGTGGTGTGGAGGAAAC | CTCCCGACAAGTTTGCACAA |
| *IL1β* | CTCTGGGATTCTCTTCAGCCA | CCTCATTGCCACTGTAATAAGCC |
| *KRT1* | TCAACAAGCGGACAAATGCA | CAATGATGCTGTCCAGGTCG |
| *OCLN* | GGGCATTGCTCATCCTGAAG | GAGTAGGCTGGCTGAGAGAG |
| *PADI4* | CAGGGGACATTGATCCGTGTG | GGGAGGCGTTGATGCTGAA |
| *PGK* | CTGTGGCTTCTGGCATACCT | AATCTGCTTAGCCCGAGTGA |

***Supplementary Figures***

******

**Supplementary figure S1: Detection of Ki67 and epithelial gene markers in RDEB-SCCs with different severity**. (A) Representative immunostaining for Ki67+ cells in in non-lesional skin (NL skin), peri-tumoral skin (PT skin), low-risk primary SCCs (Low-risk pSCC), high-risk primary SCCs (High-risk pSCC), low-risk recurrent SCCs (Low-risk pSCC) and high-risk recurrent SCCs (High-risk rSCC) in RDEB patients, scale bar = 50µm (magnification x400). Scale bar = 50µm. (B) Proportion of Ki67-positive cells compared with the total epithelial cells, n=8 for NL skin, n=11 for PT skin, n=5 for Low-risk pSCC, n=5 for High-risk pSCC, n=13 for Low-risk rSCC, n=8 for High-risk rSCC. (C) Quantitative PCR analysis of *CDH1, OCLN,* and *KRT1* transcripts in NL skin (n=8), PT skin (n=7), Low-risk pSCC (n=6), High-risk pSCC (n=3), Low-risk rSCC (n=7), and High-risk rSCCs (n=8) in RDEB patients in our study. Relative gene expression was normalized to the expression of a housekeeping gene (*PGK)* and expressed as fold-increase compared to NL skin. \* p<0.05 Kruskal Willis test with Dunn's or ANOVA with Tukey’s multiple comparison post hoc. Data are means ± SEM.

****

**Supplementary figure S2: Detection of adaptive immune** **cells in RDEB-SCCs with different severity.** (A) Representative immunostaining for infiltrated CD3+ T cells, CD4+ T cells, CD8+ T cells in non-lesional skin (NL skin), peri-tumoral skin (PT skin), low-risk primary SCCs (Low-risk pSCC), high-risk primary SCCs (High-risk pSCC), low-risk recurrent SCCs (Low-risk pSCC) and high-risk recurrent SCCs (High-risk rSCC) in RDEB patients, scale bar = 50µm. Quantification of the number of positive cells per field CD4+ T cell to CD3+ T cell ratio (C), CD8+ T cell to CD3+ T cell ratio (C), n=8 for NL skin, n=12 for PT skin, n=5 for Low-risk pSCC, n=5 for High-risk pSCC, n=13 for Low-risk rSCC, n=8 for High-risk rSCC. \* p<0.05 Kruskal Wallis test with Dunn's or ANOVA with Tukey’s multiple comparison post hoc. Data are means ± SEM.

**Supplementary figure 3*:*** **Detection of innate immune** **cells in RDEB-SCCs with different severity.** (A) Representative immunostaining for infiltrated CD163+ macrophages and tryptase+ mast cells in non-lesional skin (NL skin), peri-tumoral skin (PT skin), low-risk primary SCCs (Low-risk pSCC), high-risk primary SCCs (High-risk pSCC), low-risk recurrent SCCs (Low-risk pSCC) and high-risk recurrent SCCs (High-risk rSCC) in RDEB patients, scale bar = 50µm. Quantification of the number of positive cells per field for CD163+ macrophages (B), tryptase + mast cells (C) in the different conditions. n=8 for NL skin, n=13 for PT skin, n=6 for Low-risk pSCC, n=5 for High-risk pSCC, n=13 for Low-risk rSCC, n=8 for High-risk rSCC. \*p<0.05 Kruskal Wallis test with Dunn's or ANOVA with Tukey’s multiple comparison post hoc. Data are means ± SEM.

**Supplementary figure S4: Gene expression of inflammatory mediators involved in neutrophil activation in RDEB-SCCs by quantitative RT-PCR.** Quantitative PCR analysis of *CSF2* (A), *CSF3* (B), *IL1B* (C), *CXCL8* (D), *PADI4* (E), and *HMGB1* (F) transcripts in non-lesional skin (NL skin) (n=8), peri-tumoral skin (PT skin) (n=7), low-risk primary SCCs (Low-risk pSCC) (n=6), high-risk primary SCCs (High-risk pSCC) (n=3), low-risk recurrent SCCs (Low-risk rSCC) (n=7), and high-risk recurrent SCCs (High-risk rSCC) (n=8) in RDEB patients in our study. Relative gene expression was normalized to the expression of a housekeeping gene (*PGK)* and expressed as fold-increase compared to NL skin. \* p<0.05 Kruskal Wallis test with Dunn's or ANOVA with Tukey’s multiple comparison post hoc. Data are means ± SEM.

**(F)**

**(E)**

**(D)**

**(C)**

**(B)**

**(A)**