**Supplementary Materials and Methods**

**Differential expression analysis of cutaneous squamous cell carcinoma and basal cell carcinoma proteomic profiles sampled with e-biopsy**

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**Proteins isolation from the e-biopsy sample.**

Proteins were isolated from the e-biopsy extract using the EZ- RNA II kit (Biological Industries, Beit Haemek Ltd). Homogenizing solutions were not used in the samples; phase separation solutions were directly added as follows: 0.2 ml of water-saturated phenol, and 0.045 ml of BCP. This step was followed by protein precipitation using isopropanol and wash using guanidine hydrochloride in 95% ethanol. Air-dried protein pellets were taken for proteomic analysis as described below.

**Identifying and quantifying proteins with LC-MS/MS**

**Proteolysis**

The samples were brought to 8M urea, 400mM ammonium bicarbonate, 10mM DTT, vortexed, sonicated for 5' at 90% with 10-10 cycles, and centrifuged. The protein amount was estimated using Bradford readings. 20ug protein from each sample was reduced 60ºC for 30 min, modified with 37.5mM iodoacetamide in 400mM ammonium bicarbonate (in the dark, room temperature for 30 min) and digested in 2M Urea, 100mM ammonium bicarbonate with modified trypsin (Promega) at a 1:50 enzyme-to-substrate ratio, overnight at 37oC. Additional second digestion with trypsin was done for 4 hours at 37oC.

**Mass spectrometry analysis**

The tryptic peptides were desalted using C18 tips (Harvard Apparatus,MA), dried, and re-suspended in0.1% formic acid.The peptides were resolved by reverse-phase chromatography on 0.075 X 180-mm fused silica capillaries (J&W) packed with Reprosil reversed-phase material (Dr. Maisch GmbH, Germany). The peptides were eluted with a linear 180-minute gradient of 5 to 28%, 15 minutes gradient of 28 to 95%, and 25 minutes at 95% acetonitrile with 0.1% formic acid in water at flow rates of 0.15 μl/min. Mass spectrometry was performed using Q-Exactive Plus mass spectrometer (Thermo Fischer Scientific, CA) in a positive mode using a repetitively full MS scan followed by collision-induced dissociation (HCD) of the 10 most dominant ions selected from the first MS scan.The mass spectrometry data from all the biological repeats were analyzed using the MaxQuant software 1.5.2.8 vs. the human proteome from the UniProt database with 1% FDR. The data were quantified by label-free analysis using the same software, based on extracted ion currents (XICs) of peptides enabling quantitation from each LC/MS/MS run for each peptide identified in any of the experiments.

**Numerical simulations of electric fields distribution in the skin tissue and electric field-induced thermal effects.**

To model the distribution of the electric fields in the skin with tumors during e-biopsy, we used the finite elements method (FEM), which allows us to find an approximate solution in complex geometries for solving the Laplace differentiation equation with boundary conditions defined by the applied voltage. Numerical solutions for a Laplace equation that result in the electric field distribution in the brain and brain melanoma models were performed in QuickField (Terra Analysis, Denmark). The electric and thermal properties of tissues used to appear in **Table S1.** The model files with full solutions appear online at the following link: <https://github.com/GolbergLab/BCCvsSCC.git>

The model constitutes of a 2D mesh, in a plane-parallel mode and the problem type is direct current (DC) conduction. A general carcinoma lesion (the electrical1,2 and thermal3–5 properties of BCC and cSCC are similar) was modelled as a 2D rectangle with 20mm length, 2mm infiltration depth, and surrounded with a healthy skin strap of 5mm length from both sides of the lesion. Needle electrode with radius of 0.3mm is positioned 10mm from the ground electrode and it penetrates the skin 1mm into the lesion. Ground electrode is placed without skin penetration on the lesion surface area, with 3mm radius.

Electric field distribution is mainly determined by the electroporation protocol, such as pulse parameters, electrode configuration and physical features of the tissue. For simplicity, each layer in this model is assumed to have homogeneous and isotropic properties, taking into consideration the complexity of the skin layers as the stratum corneum, epidermis and dermis, all with anisotropic dielectric properties6, including lesion, that paved its way through skin layers, infiltrating fat layer.

We assume the thermal properties of the skin didn't change after electroporation7, while the electric conductivity after electroporation increased 8. Direct current (DC) conduction and steady-state heat transfer problems were coupled with transient heat field problems.

In steady-state heat transfer, with Dirichlet boundary conditions, the temperature is constant with time: TAirline = 25 ℃, where the airline differentiates between skin and air.

Heat sources were imported from DC conduction and steady-state heat transfer problems coupling, for the thermal field problem. To calculate the power supplied by the pulsed electric field, we used the following equation (**Eq. 1**):

|  |  |
| --- | --- |
|  | (**Eq. 1**) |

where*(W)* is the total average power delivered by square pulse electric field, R (ohm) is the resistance, is the root mean square voltage, *V (Volt)* is the applied voltage, is the duration of the pulse and  *(Hz)* is the frequency of the pulse wave.

To calculate the electric field distribution, we used the Laplace equation (**Eq. 2**):

|  |  |
| --- | --- |
|  | (**Eq. 2**) |

With the following potentials: , , and .

To calculate the thermal distribution, we solved the transient heat transfer equation (**Eq. 3**):

|  |  |
| --- | --- |
| = | (**Eq. 3**) |

Where *T* is the temperature (*K*), (W is the thermal conductivity, is the specific heat capacitance, *t* (s) is time, *q* () is the volume power of heat sources. In our problem *q* is the average volume power supplied by a pulsed electric field. We assume that heat is transferred by convection between the air, and skin, and the convection coefficient with air is *α =* 5*(W K-1m-2)* 9

**Reproducibility analysis**

To assess the reproducibility of e-biopsy methodology, the similarity between the measurements gathered from 3 sampled patient’s locations was estimated. Our assumption is that the actual proteomes in the sampled locations should be very similar, given these locations are spatially and phenotypically close. Therefore, we expect that protein profiles sampled by a reliable technology to be in a high agreement with each other. Together with this, inherent tissue spatial heterogeneity would prevent even from the ideal sampling method to receive the exact measurement replicas.

Specifically, to assess the similarity of the samples produced by e-biopsy method, while reducing the impact of local spatial heterogeneity, we calculated maximal intra-patient Pearson (*scipy.stats.pearsonr*) correlation between each of 3 pairs of measured raw protein intensities. The per-patient results are available in **Table S2**.

**Calculating FDR for condition-unique proteins**

To assess the FDR for the number of proteins identified as *unique* in a certain condition, we calculated the probability to observe a protein uniquely in this condition for the predefined number of times (or more) by a mere chance. Specifically, the probability of a certain protein *t* to appear in at least 4 (and at most 60) BCC samples, while to never appear in any of 63 cSCC samples (and vice versa – at most 63 cSCC and never in 60 BCC samples) by a mere chance was 2.9e-03 (3.6e-03 in the opposite direction). This is derived as follows (**Eq. 4**), where *HG(123,k,60,k)* is a hypergeometric probability of selecting *k* out of *k* samples inside the subgroup sized 60 in population sized 123; and is calculated from data:

|  |  |
| --- | --- |
|  | (**Eq. 4**) |

This leads to the expectation of 20.3 such proteins (25.8 in the opposite direction), resulting in FDR of 2.51e-01 for 81 such proteins uniquely observed in BCC and of 2.51e-01 for 103 such proteins uniquely observed in cSCC samples. On the patient level, the probability of a certain protein to appear in at least 3 (and at most 20) BCC patients, while to never appear in any of 21 cSCC patients (and vice versa – at most 21 cSCC and never in 20 BCC patients) by a mere chance is 7.5e-03 (3.8e-03 in the opposite direction) based on calculations is similar to **Eq. 4** and leading to the expectation of 53 such proteins (27.2 in the opposite direction). This results in FDR of 5.76e-01 for 92 such proteins uniquely observed in BCC and of 4.94e-01 for 55 such proteins uniquely observed in cSCC patients.

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