Ulceration in Mexican Acral Lentiginous Melanoma patients.

Minor research project report

Ivar van der Zee

5693225

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Supervisors:

Dr. C. Daniela Robles-Espinoza, National Autonomous University of Mexico

Irving Simonin, National Autonomous University of Mexico

Examiner:

Dr. ir. Jeroen de Ridder, University Medical Center Utrecht

Abstract

Objectives: Identify biomolecular mechanisms responsible for acral lentiginous melanoma (ALM) ulceration and its associated worsened clinical outcomes.

Methods: Transcriptomes of 59 Mexican ALM patients with varying ulceration status were analysed through the Feature-Engine Python library, which ranked genes based on how much their expression differs between ulcerated and non-ulcerated tumours. This ranking was analysed through gene set enrichment analysis to identify biological components.

Results: Multiple proteins that make up desmosomes (a cell-cell adhesion complex) appear downregulated in ulcerated tumours. The genes PERP and TP63, which are thought to be essential to desmosome formation, also appear downregulated.

Conclusion: Given the role desmosomes play in both skin integrity and tumour suppression, we hypothesize them to be the link between ulceration and the associated worsened clinical outcomes. PERP could be involved in causing their dysregulation.

Layman's summary

Acral Lentiginous Melanoma (ALM) is a type of skin cancer occurring on the hairless skin found on your hands and feet, it is the most common type of melanoma in several countries in Latin America and Asia. However, because most cancer research is focussed on European descent populations —in which ALM is not a big issue— ALM has remained understudied compared to other forms of skin cancer. One specific phenomenon that remains poorly understood is so-called 'ulceration', which is the development of a sort-off open blister on top of the tumour which occurs in roughly 40% of ALM cases. We know that patients that have such ulcerated tumours suffer from worse disease progression and a lower chance of survival compared to ALM patients with non-ulcerated tumours. However, we don't know why some tumours ulcerate while other don't and how ulceration relates to lower chances of survival. Because ALM ulceration is linked to worsened disease progression, a good understanding of it could help us develop better treatment for those that suffer from ulcerated ALM. Therefore, this research compared both ulcerated and non-ulcerated tumours from 59 Mexican ALM patients to try and identify how ulceration works and why it worsens disease progression.

The way we did this is by measuring the expression level of every single gene in each tumour through a technique called RNA-sequencing. Comparing these expression levels between ulcerated and non-ulcerated samples allowed us to determine which genes are more active or less active in ulcerated samples compared to non-ulcerated ones. Combining this information with existing public knowledge about the function of these proteins (a process called 'Gene Set Enrichment Analysis') allows us to gain insight into what biological processes might be involved in ulceration. Simply put, if many proteins of which we know they are important for process X also turn out to show different expression levels between ulcerated and non-ulcerated samples, then process X might be involved in ulceration.

We found that certain proteins which help glue cells together and organize them were less active in ulcerated tumours. These proteins normally form a sort-of anchor called a 'desmosome' that can strongly connect two cells together. However, in ulcerated tumours these desmosomes seem dysregulated. Desmosome dysregulation has been linked to worsened disease progression in many different cancers since cancer generally benefits from a decrease in the cell organization and adhesion that desmosomes normally provide. Furthermore, it could explain ulcer formation as well, since the outer layer of skin (which is affected by ulceration) requires desmosomes to maintain its strength. What causes desmosome dysregulation in ulceration ALM in the first place also remains uncertain. We hypothesise that loss of the 'PERP' gene might play a role, as its loss could explain the worsened disease progression as well as desmosome dysregulation.

Since this analysis alone is not enough to draw definite conclusions about ALM ulceration, we suggest our results to be considered as pointers for future research. Most importantly, we suggest future research to look at the type and size of each ulcer instead of only determining whether a tumour is ulcerated or not, as this would allow for more detailed analyses and comparisons between patients.

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Introduction

Acral lentiginous melanoma (ALM) is one of five major histological subtypes of melanoma. Its characterized by its lentiginous (radial) growth pattern and its location on acral glabrous skin, which exists on hair-free portions of the hands and feet (1). Worldwide, the proportion of ALM as a total of melanoma cases varies widely across ethnicities. In European-descent populations it constitutes around 3% of melanomas, whereas in Asia and Latin-America it accounts for up to 40% (2). Given the rarity of ALM in European-descent populations, it has historically been less of a priority within melanoma research compared to other subtypes. As a result, the aetiology of ALM remains poorly understood to this day. In recent years, however, research efforts surrounding ALM within non-European ethnicities have intensified to address this gap in understanding.

One aspect of ALM currently under scrutiny is ulcer formation on the tumour, or ulceration. Although no evidence- or consensus-based definition of melanoma ulceration exists, it is generally defined as loss of the epidermal matrix characterized by skin discontinuity over the tumour site (Figure 1); It can differ in type (i.e., infiltrative or attenuative) and extent to which it covers the tumour (3). Currently, no large-scale studies into the prevalence of ulceration in ALM exist; however, recent studies suggests it occurs in roughly 40% of cases (4–6). When it does, prognosis is worsened, as patients with ulcerated melanoma show up to 50% lower five-year melanoma specific survival (3) and increased sentinel lymph-node positivity (7). Accordingly, ulceration status is incorporated into the ACJJ melanoma staging system (8). However, the applicability of conventional melanoma staging to acral melanomas like ALM is controversial, with one recent study concluding that —in contrast to conventional staging— ulceration loses its prognostic value in thick (>3 mm) acral melanomas (9). Still, the same study concludes that despite some uncertainties, ulceration in ALM nonetheless has an undeniable correlation with worsened prognosis.

Though the prognostic implications are quite clear, we lack understanding of the molecular mechanisms behind why some ALMs ulcerate while others do not, and why ulcerated ALMs show worse outcomes than otherwise similar non-ulcerated ALMs. Existing literature on this subject is limited and, so-far, inconclusive (10–15). Most studies consider ulceration to be a phenotypic indicator of underlying molecular changes that, besides ulceration, cause worse clinical outcomes (10,13,15). However, they fail to identify what changes these are, or what pathways they affect. More importantly, these studies consider cutaneous melanoma as whole, without considering individual subtypes like ALM. This limits their applicability to ALM, since ALM has distinct molecular features compared to other melanomas and is localized to a unique tissue type (i.e., glabrous skin) (1), which could very well affect ulcer development.

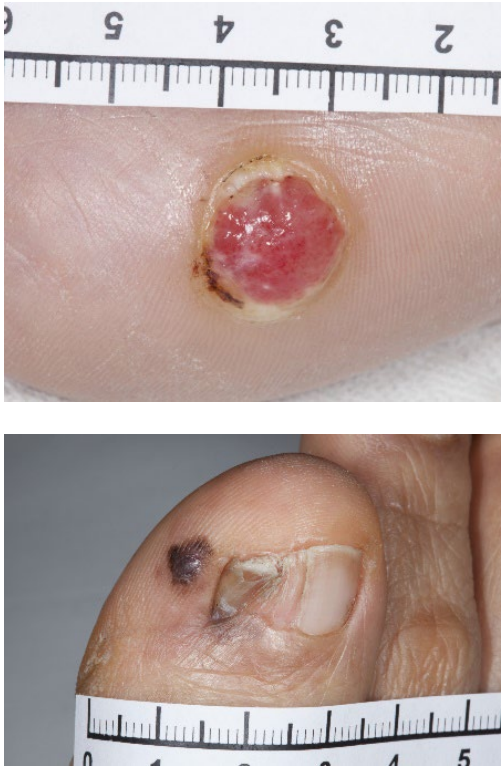

Figure 1: Photographs of ulcerated (a) and non-ulcerated (b) acral lentiginous melanoma localized near the heel pad (a) and on great toe (b). Figure source: Howard et al., 2020 (6)

Given the gap in understanding and negative prognostic implications of ALM ulceration, it is evident that further research into its causes and effects could advance our understanding of ALM and potentially contribute to better treatment options. Therefore, this study aims to identify molecular mechanisms possibly involved in ALM ulceration so as to provide a starting point for further research. To achieve this, genomic and transcriptomic tumour sample data collected from 59 Mexican ALM patients were analysed to find discriminant features that separate ulcerated from non-ulcerated cases.

Methods

Data collection

The data used in this study is part of a larger ALM research effort led by the Cancer Genetics & Bioinformatics lab of the National Autonomous University of Mexico, which collected Formalin-Fixed Paraffin-Embedded (FFPE) tumour samples from Mexican ALM patients at the National Cancer Institute in Mexico (Instituto Nacional de Cancerología, INCAN) and the High Specialty Regional Hospital of the Bajio (Hospital Regional de Alta Especialidad del Bajio, HRAEB), under ethics agreements INCAN/017/041/PBI and CI/HRAEB/2019/053 in Mexico and NHS 18/EE/0076 in the United Kingdom.

On 113 of these, exome capture transcriptome sequencing (HiSeq 4000) was performed by the Wellcome Trust Sanger Institute. Of these 113 samples, 60 were primary tumour samples, passed quality control, and had ulceration data available; these 60 were included in this study. They were collected from 59 patients, whose clinical characteristics are outlined in table 1.

Table 1: Clinical characteristics of included acral lentiginous melanoma patients.

Characteristic	Ulceration	No Ulceration	Total
N (%)	35 (59.3)	24 (40.7)	59
Age, years			
Mean (SD)	61.9 (12.7)	57.5 (10.9)	60.1 (12.1)
Median (IQR)	60.0 (55.0 – 70.0)	60.5 (50.8 – 63.0)	60.0 (52.5 – 66.5)
< 65	22 (52.4)	20 (47.6)	42 (71.2)
≥ 65	13 (76.5)	4 (23.5)	17 (28.8)
Sex			
Male	15 (78.9)	4 (21.1)	19 (32.2)
Female	20 (50.0)	20 (50.0)	40 (67.8)
Primary site			
Sole	25 (61.0)	16 (39.0)	41 (69.5)
Nailbed	6 (46.2)	7 (53.8)	13 (22.0)
Other	4 (80.0)	1 (20.0)	5 (8.5)
Stage (ACJJ 8th ed.)			
0	0 (0.0)	3 (100.0)	3 (50.8)
I	3 (21.4)	11 (78.6)	14 (23.7)
II	13 (76.4)	4 (23.5)	17 (28.8)
III	17 (77.3)	5 (22.7)	22 (37.3)
IIII	2 (66.7)	1 (33.3)	3 (5.1)

Data analysis

Analysis of tumour transcriptomes was performed on pre-existing TPM normalized counts generated by HTseq version v0.7.2 (16) using alignments made through STAR version 2.5.0c (17) against human reference genome 38 (18) and ERCC 92. These counts were provided by Estefania Vasquez, a PhD student working on the same project. Before running any analysis, 15 thousand genes with one or less non-zero values across samples were removed as these contain no useful information to this study. Subsequently, the variances of the remaining 47K genes (including isoforms) were stabilized by $\log_2$ transforming all counts. Genes that differentiate between ulcerated and non-ulcerated were identified through the SelectBySingleFeaturePerformance (SBSFP) function from the FeatureEngine python library version 1.5 (19). One of the reasons we opted for this approach was that more traditional methods such as DESeq2 did not yield useful results, likely as a result of low sample size. SBSFP is a feature selection method that trains and assesses a model for every single feature separately, thereby determining which features hold predictive power for a certain outcome and which do not. In case of this analysis, SBSFP trains a separate logistic regression that predicts ulceration status for every single gene. These models are subsequently evaluated by calculating their c-statistic (roc-auc) score which thus represents the extent to which the expression of each gene is predictive of a sample's ulceration status. Simply put, the higher a gene's roc-auc score the better it differentiates between ulcerated and non-ulcerated samples. Since our sample size is relatively small, train-test set randomization can have a big effect on the c-statistic. To circumvent this, every gene is assessed through twelve random four-fold cross validations, meaning the score assigned to a gene is the average of 48 individual scores. A reproducible and annotated version of the analysis is provided in an accompanying Jupyter notebook.

Enrichment

To interpret the SBSFP scores and understand what biological components relate to ulceration status, we perform enrichment analysis. For this study, pre-ranked gene-set enrichment analysis (GSEA) is most suitable, as it relies on a user-supplied ranked list of genes which is exactly what SBSFP provides as it assigns every gene an individual score based which can thus be ranked. GSEA looks at how genes belonging to a-priori defined sets (e.g., Gene Ontology terms) are positioned within the user-provided ranking of all genes. If the genes belonging to a certain set (e.g., amino acid binding or cell adhesion) are normally distributed within the ranking, this set is considered unrelated with the phenotype on which the gene ranking was based. Conversely, if genes belonging to a certain set are significantly skewed towards the top the ranking, this indicates an association between that set and the phenotype. Simply put, GSEA allows us to understand what biological components or pathways appear related to ulceration status by looking at how their genes are distributed within our gene ranking.

The advantage or GSEA over a more straightforward overrepresentation analysis (ORA) of the genes with a high SBSFP score is that GSEA considers every gene's position within the ranking, whereas ORA would require an arbitrarily chosen cut-off which could result in terms of which all genes are only slightly associated with ulceration not being picked up as their individual scores would not make the cut-off.

Pre-ranked gene-set enrichment analysis (GSEA) was performed on the SBSFP gene ranking through the BlitzGSEA (20) python package with Gene Ontology Cellular Component 2021 terms containing <75 genes (21,22).Terms with >75 genes were filtered out as these are broad and generally uninformative to this study. For example, large terms like 'tissue development' or 'skin', which could very well be involved in ulceration but are too general to lead to novel insights.

Cross-check against other -omics data

Copy-number variation (CNV) and mutation data were available in addition to RNAseq data for 35 and 47 of the 60 samples, respectively. To see whether genes separating ulcerated for non-ulcerated in terms of expression also show separation in terms of mutation rates, enriched GO terms with FDR<0.01 from the initial transcriptome analysis were cross-checked against mutation data. For these, the fraction of ulcerated and non-ulcerated samples that had a mutation in at least one of the genes belonging to each term was calculated and compared. CNV comparison was performed more broadly since just 13 non-ulcerated samples with CNV data were available. Pre-existing Gistic2 output files were parsed to extract each amplification and deletion regions identified by Gistic2 along with the copy-numbers of those regions for each individual sample. For each region, the number of ulcerated and non-ulcerated samples with a deviating copy-number in that region were compared. This allows us to see whether any of these region's copy number differs based ulceration status and, if so, whether genes in that region correspond to any of the enriched GO terms.

Results

SelectBySingleFeaturePerformance

Roc-auc scores for the 46,625 analysed genes ranged from 0.299 to 0.816, where 1 represent a model that predicts all test-set samples correctly, 0.5 represents a model that is on-par with random guessing, and 0 represents a 'perfectly imperfect' model. Note that while a score of 0.816 requires a gene to differentiate based on ulcerated status, a minimum score of 0.299 is more likely caused by a gene unrelated to ulceration whose model turned out worse than random guessing (0.5) by chance since roc-auc variance increases when no real differentiation is present. The distribution of roc-auc scores is provided in Figure 2.

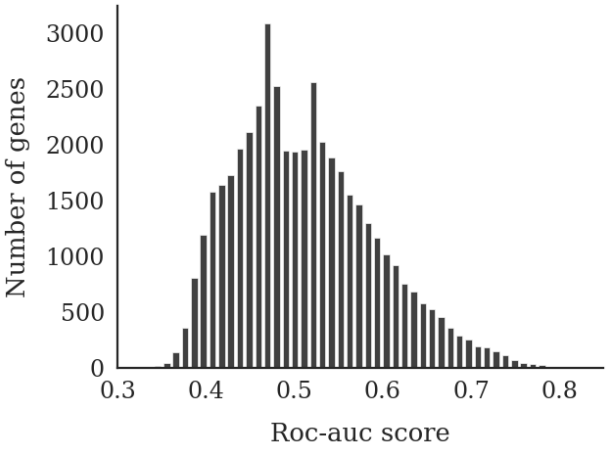

Figure 2: Roc-auc score distribution. The figure shows how the roc-auc scores of individual genes are distributed. The vast majority of genes fall below 0.7 and hence show minimal to no correlation to ulceration status.

To provide an idea of what expression distributions result in which roc-auc scores, Figure 3 provides an overview of 6 genes with scores of roughly 0.8, 0.65, and 0,5. It illustrates that genes with scores of 0.65 and below show minimal differentiation between ulceration status, meaning the vast majority of genes their expression patterns appear unrelated to ulceration and only the top 600 (or top 1.2%) appear to exhibit any differentiation. Insight into what cellular components are enriched at the top of the roc-auc scores is provided by the GSEA results.

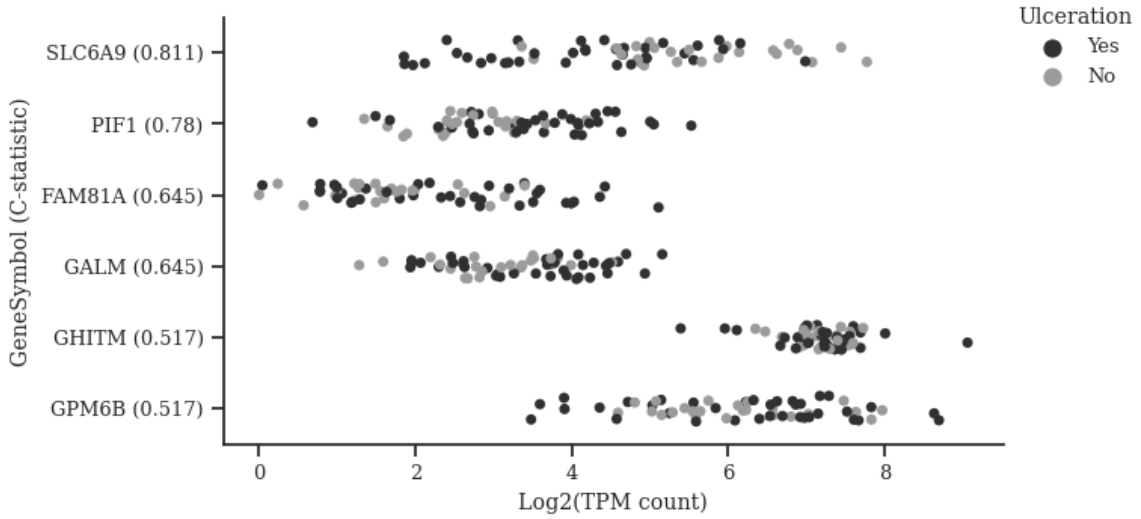

Figure 3: Expression levels of 6 genes with differing roc-auc scores. This figure illustrates how different expression levels result in different roc-auc scores, it shows 6 genes in 3 broad categories of roc-auc scores. Note how the better the expression levels are separated according to ulceration status, the higher the resulting score and vice-versa.

GSEA Enrichment

GSEA analysis found 18 enriched terms with an FDR < 0.01, of which the 10 with the highest normalized enrichment score (NES) are outlined in Table 2. The genes belonging to each of these are available in appendix 1. The cornified envelop —a tough outer layer of skin— stands out as it shows the most enrichment by some margin. However, this should be considered more of a sanity check than a meaningful result since the cornified envelope is precisely what's lost during ulceration (Figure 1), so differentiated expression of this term is to be expected. None of the enriched terms showed similar differentiation in terms of mutation or copy-number variation data.

Since a deep-dive into each of these terms would be too time-consuming for this project and lead to an overly lengthy report without a well-defined scope, this report will solely focus on desmosomes and their potential role in ulceration causes and effects within ALM. This choice was mainly based on a surface level literature review of each enriched term. Expression levels of genes that make up desmosomes are provided in Figure 4.

Table 2: Top 10 enriched terms by normalized enrichment score.

Term name	Term ID	Number of genes in set	Normalized enrichment score	FDR
Cornified Envelope	GO:0001533	43	7.50	2.65e-11
Condensed Chromosome	GO:0000793	53	4.43	9.83e-4
Mitochondrial Intermembrame Space	GO:0005758	57	4.29	1.51e-3
Organelle Envelope Lumen	GO:0031970	63	4.23	1.61e-3
Respiratory Chain Complex I	GO:0045271	41	4.17	1.63e-3
Mitochondrial Respiratory Chain Complex I	GO:0005747	41	4.17	1.63e-3
Intermediate Filament	GO:0005882	50	4.09	1.81e-3
Desmosome	GO:0030057	17	4.00	2.43e-3
CMG Complex	GO:0071162	10	3.91	3.18e-3
Spindle Microtubule	GO:0005876	61	3.73	5.49e-3

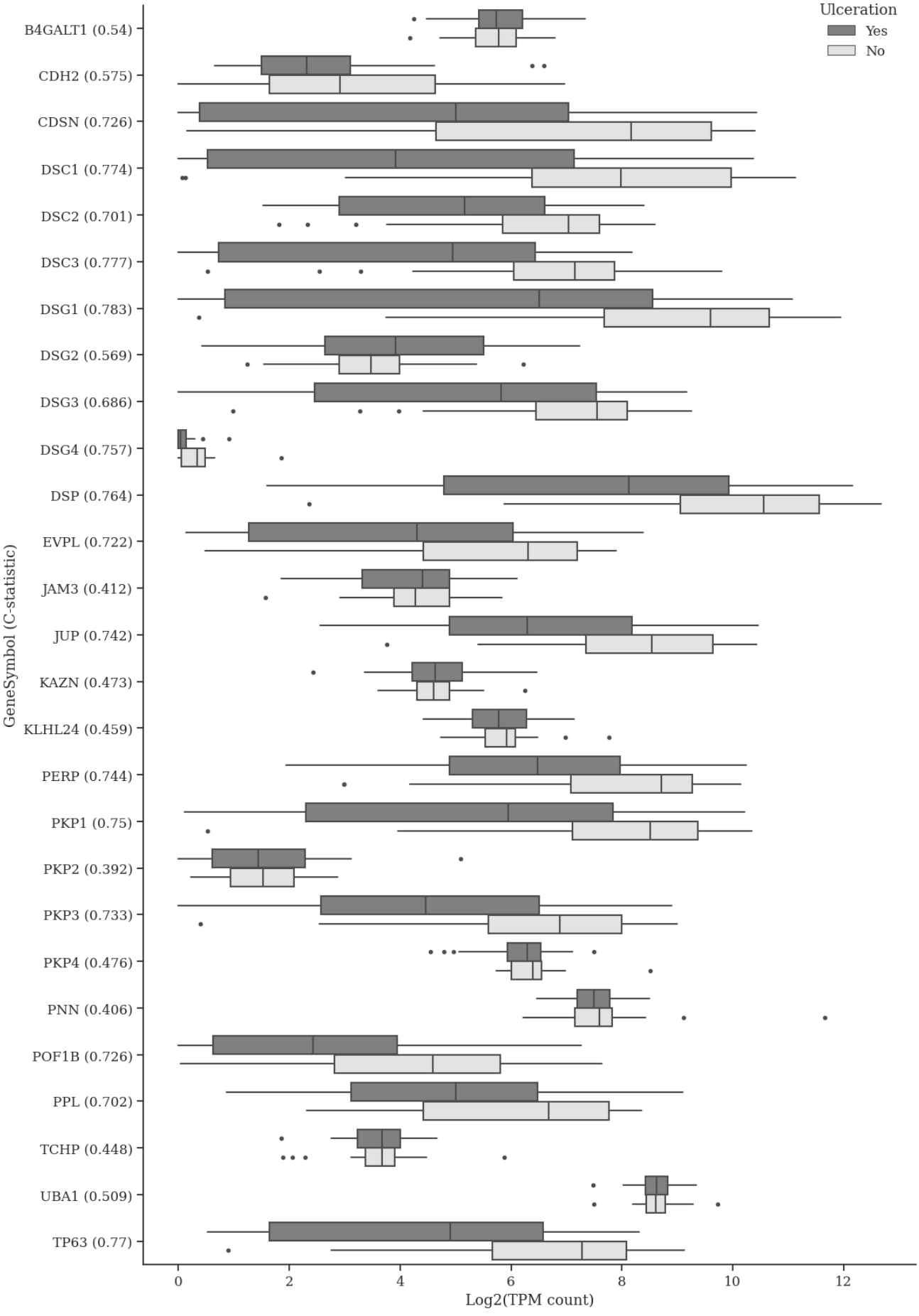

Figure 4: Expression levels of TP63, PERP, and the genes that make up desmosomes (GO:0030057) by ulceration status.

Desmosomes in oncogenesis

Given the importance of cell-cell adhesion in melanoma (23) and the apparent difference in desmosome expression between ulcerated and non-ulcerated samples, it is worth looking into the function and the potential roles desmosomes have within ALM ulceration. Therefore, this section aims to provide an overview of what desmosomes are, how they function in healthy tissues, what consequences their dysregulation could have, and what could cause their dysregulation in ulcerated ALM.

Desmosomes are intercellular anchoring junctions that provide strong cell-cell adhesion, thereby providing resilience to tissues that experience intense mechanical stress like the epidermis, bladder, or heart. Furthermore, they are similar to other cell-cell junctions in that they act as signalling platforms for the regulation of cell proliferation, differentiation, migration, morphogenesis, and apoptosis (24–26). Desmosomes are primarily composed of 3 protein families: Desmosomal cadherins, armadillo proteins, and plakins. Desmosomal cadherins comprise of desmocollins (DSC1-3) and desmogleins (DSG1-4), these are the membrane-spanning parts of desmosomes that mediate adhesion. Armadillo proteins constitute the intracellular binding partners of the aforementioned cadherins, they include plakoglobin (JUP) and plakophilins (PKP1-4). From the plakin family, desmoplakin (DSP) is most important as it interacts with JUP with and intermediate filaments, thus connecting desmosomes to the cytoskeleton. An overview of this arrangement of proteins is provided in Figure 5.

Within this study, all three desmosomal protein families show differing expression patterns between ulcerated and non-ulcerated samples (Fig. 4). Ulcerated samples show lower and more variable expression levels for all desmocollins (DSC1-3), most desmogleins (DSG1, 3, 4), plakoglobin (JUP), desmoplakin (DSP), and certain plakophilins (PKP1, 3). A possible explanation for why DSG2 and PKP2 do not show the same differentiation is that they are less localized to the outermost layers of skin (25) (Figure 5), where ulceration occurs. All in all, this suggests some degree of increased desmosome dysregulation occurs in ulcerated compared to non-ulcerated ALM.

Before, desmosome dysregulation was mainly thought to cause degenerative diseases like palmoplantar keratoderma while adherens junctions were related to cancer. Recently, however, desmosomes are increasingly being considered relevant to tumour proliferation and metastasis (24). The general idea behind this relationship is that the cell-cell adhesion facilitated by desmosomes is crucial for proper tissue homeostasis while desmosome dysregulation upsets this balance, which reduces tumour suppression and allows parts of tumours to break off and spread more easily (23). Still, the exact effects of desmosome dysregulation are not completely understood and studying it is proving difficult; In-vivo loss-of-function studies are hard given lethality of desmosome deficiency in mice and expression levels from human tumours are conflicting (24). For instance, low expression of DSG1, DSC2, DSC3, DSG3, JUP, PKP1-3, and DSP has been associated with worse outcomes in skin cancer, yet upregulation of DSG2, DSG3, PKP1, and PKP3 was also found to correlate to increased tumour proliferation (27). This suggests desmosomes have multiple ways of interacting with cancer through both under- and overexpression. Still, most evidence related to epithelial cancers —including this study— suggest lower expression levels of desmosomal proteins correlate to worse clinical outcomes (23–26).

Besides the exact effects of desmosome dysregulation, its causes remain unclear as well. One gene of interest is p53 apoptosis effector related to PMP-22, also known as PERP. A tetraspan plasma membrane and transcriptional target of p53 and p63 (28), of which the latter is responsible for morphogenesis in stratified epithelia (29) and shows lower expression in ulcerated samples similarly to PERP (Figure 4). Although the function of PERP is as of yet not completely understood, downregulation of PERP was found in several cancers and it appears essential to desmosome function through interaction with JUP and DSP (28). A 2005 study found $PERP^{-/-}$ mice die during, or right after birth with severe

blistering (12), whereas DSC1 or DSG3 knockout mice were shown to survive, albeit with epidermal integrity defects (30,31). This suggests PERP is just as essential to desmosome function as the main desmosomal components, if not more. Another relevant study induced PERP deficiency in the stratified epithelia of a squamous cell carcinoma mouse model through conditional knockout (11). It showed PERP deficient mice developed tumours earlier and more often while having downregulated desmosomes compared to controls. Interestingly, the study showed adherens junctions remained intact while desmosomes were downregulated. The authors therefore suggest *"desmosome loss is a specific event important for tumorigenesis rather than a reflection of a general change in differentiation status"* (11).

Besides being essential to skin development through regulating desmosomes, a 2020 review concluded that PERP acts as a tumour suppressor independently of desmosomes as well (28). They propose that besides dysregulating desmosomes, PERP loss results in impaired apoptosis and recruitment of inflammatory cells, which would mean that should desmosome dysregulation in ALM indeed be caused by PERP loss, then desmosomes would likely not be the only source of resulting disease progression. More importantly, however, PERP's double-role in tumour suppression and skin development via desmosomes could explain the relationship between ulceration and worsened clinical outcomes, as PERP loss would be able to cause both.

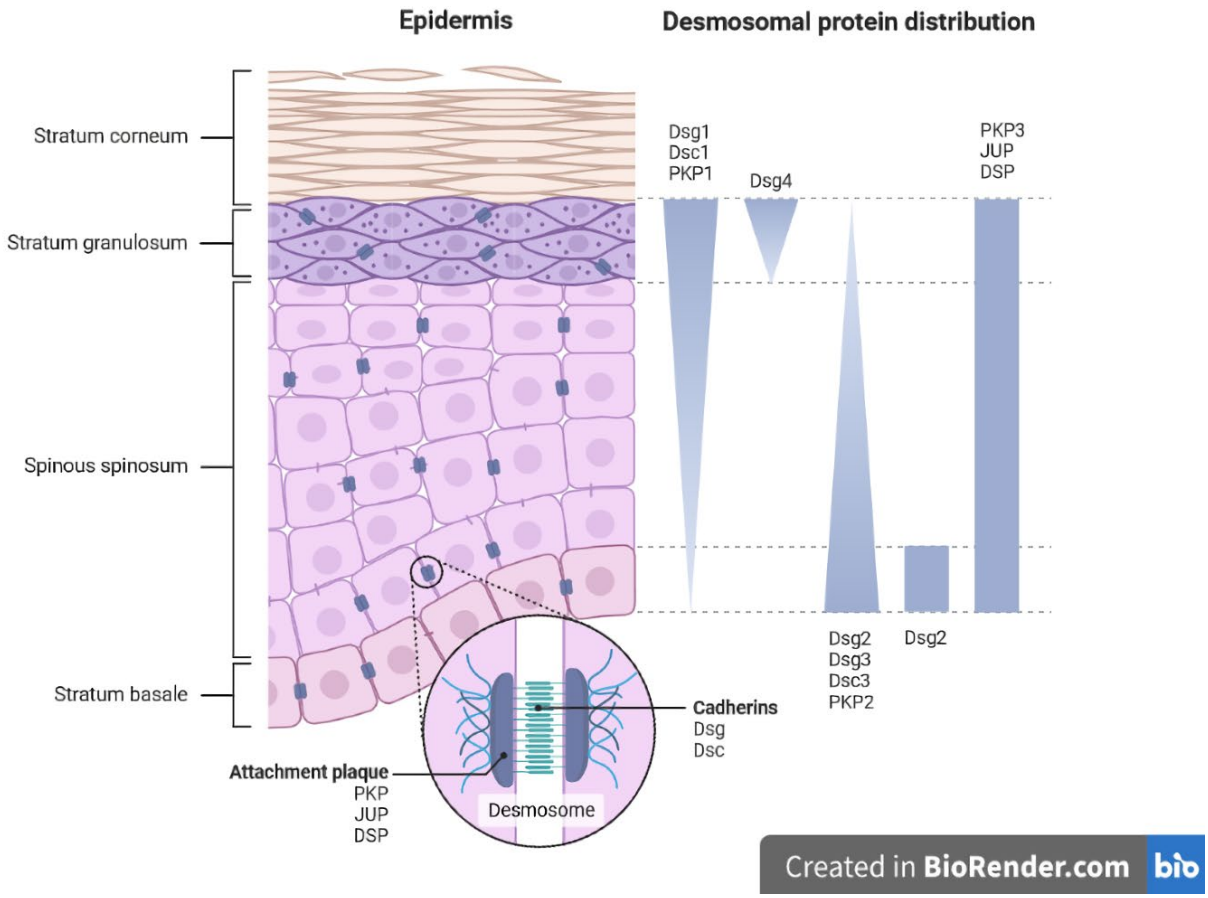

Figure 5: Illustration shows desmosomes facilitating cell-cell adhesion in the epidermis, how desmosomal proteins are organized within a desmosome, and how subtypes of desmosomal proteins are localized across the epidermis. Illustration adapted from a BioRender.com template.

Discussion

This study analysed RNA expression levels, copy-number variations, and mutation data of 59 Mexican ALM patients by ordering genes based on how well their expression level separates samples based on ulceration status and subsequently performing GSEA analysis. Among other things, expression levels suggest that desmosomes are dysregulated in ulcerated ALM. In light of existing evidence relating desmosome dysregulation to cancer proliferation, this could (partly) explain why clinical outcomes of ulcerated tumour are worse.

The main limiting factor of this study is its sample size of 60 samples. This likely limited the use of traditional tools like DESeq2 to conclusively determine whether genes are differentially expressed and prevented the detection of small but significant differences in expression levels between ulceration status. Because the limited sample size did not allow us to effectively control for differences in stages or other clinical characteristics besides ulceration, another limitation arose from differences in average pathological stage between ulcerated and non-ulcerated samples (table 1). Part of this bias is caused by ulceration status itself, since ulceration status is considered in staging and can cause patients to move from stage I to II (8). However, ulceration status is not considered when upstaging from stage II to III and thus did not cause the elevated number of stage III patients in the ulcerated group. Therefore, we are not purely comparing samples that differ in terms of ulceration status, but also comparing differences in terms of disease progression. While this is very hard to avoid, it could have us relating effects to ulceration while they might just be effects of general disease progression. Besides these limitations regarding the cohort size and comparability, the binary nature of the available data (i.e., yes/no) also proved limiting because ulceration in cutaneous melanoma is heterogenous in terms of both type and extent (3). Like other research into melanoma ulceration (9,13), our dataset contains a yes or no description of ulceration while not every ulceration event is the same. This absence of the more detailed data potentially limits meaningful analysis or interpretation.

While these limitations prevent any hard conclusions from being drawn about the role of desmosomes and PERP in ALM ulceration, the results do allow us to hypothesize about a possible role for PERP in explaining the link between ulceration, desmosomes, and worse prognosis in ALM. Seeing as PERP plays a role in both tumour suppression and skin homeostasis via desmosomes (11,24,28), it could explain why ulceration and worse disease progression occur in tandem. After all, ulceration is a failure of skin homeostasis while dysregulated desmosomes and decreased tumour suppression cause worsened clinical outcomes. Our results back this up as expression levels of desmosomal proteins and PERP appear decreased in ulcerated samples, suggesting PERP could be the missing link in relating ulceration to tumour progression and worse prognosis.

The cause of PERP loss could lie with p63, as it is considered a 'master-regulator' of stratified epithelium and a proven regulator of PERP in stratified epithelium (29). However, since there are many things able to disorganize epithelial cells and the exact role of PERP itself is as of yet unclear, we suggest further research to be centred around validating the role of desmosomes and PERP in ulcerated ALM in favour of digging into the precise cause of their lower expression levels.

Further research would greatly benefit from comparing samples taken before, during and after ulceration as this would allow for insight into the ulceration process. However, this is challenging given most cases of ulcerated ALM are diagnosed post-ulceration and non-ulcerated are treated to, among other things, prevent ulceration. After all, letting tumour ulcerate during treatment is highly unpreferable. Instead, a study wherein data concerning the type (i.e., infiltrative or attenuative) and extent (i.e., percentage of tumour surface) of ulceration is collected might prove more feasible while also allowing for finer comparison between different ulcerated ALMs. Furthermore, a tumour does not ulcerate overnight. Instead, the epidermal matrix

is lost gradually. Therefore, collecting data on the stratified epithelia of all samples regardless of ulceration status could provide additional insight as there might be cases where the epidermal matrix is affected in a way other than simply disappearing. Besides the primary tumours, comparing distant metastasis of tumours with differing ulceration status might also prove informative, Since this would allow for comparisons outside of the context of acral skin.

Although it is too early to point to any concrete improvement options in ALM treatment, some ideas did arise from this analysis. For instance, given the current uncertainties around ulceration in ALM staging (9) and by extent treatment choice, it could prove beneficial to integrate information about the molecular changes responsible for ulceration —like perhaps desmosome/PERP expression— into diagnostics and staging. This could allow for more precise prognosis and staging of disease severity without relying on a blunt

yes-no statement about ulceration. Especially since ALM is often localized to places prone to mechanical stress like the foot, which could very well have an impact the likelihood of ulceration without being indicative to disease severity. Besides that, in case loss of a specific gene like PERP ultimately turns out to be responsible for ulceration and worsened outcomes, then PERP reactivation therapy could prove a fruitful avenue to explore. Of course, for the time being such predictions remain shrouded in uncertainly. However, they do illustrate that furthering our understanding of ALM ulceration is important, as it could very well result in tangible therapeutic benefits for the ever increasing numbers of patients suffering from ALM.

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Appendix

Appendix A: Genes belonging to top 10 enriched Gene Ontology Terms

Term name	Term ID	Number of genes in set	Genes
Cornified Envelope	GO:0001533	43	KRT1, DSP, ANXA1, CDSN, JUP, KRT2, KAZN, HRNR, KRT10, CNFN, LCE1D, FLG2, PKP3, DSG1, PKP2, DSG2, DSG3, LORICRIN, PKP4, DSG4, SPRR1A, DSC1, SPRR1B, DSC2, PKP1, DSC3, IVL, SPRR2F, SPRR2G, FLG, SPRR3, CSTA, TCHH, PPL, EVPL, SCEL, RPTN, TGM1, PI3, SPRR2A, SPRR2B, SPRR2D, SPRR2E
Condensed Chromosome	GO:0000793	53	CBX3, LIG4, P3H4, SMC1A, SMARCA5, LRPPRC, CENPE, NSMCE4A, EID3, SGO1, INCENP, FANCD2, DMC1, L3MBTL1, SYCP2L, SUV39H1, NCAPG, HMGB1, HMGB2, CTCF, MEIKIN, NOL6, AURKC, AURKB, CHEK1, SHOC1, RRS1, ESRRB, BRD4, XRCC4, NSMCE3, NSMCE2, NSMCE1, RAD50, TUBG1, SETMAR, RAD51, NCAPD2, NCAPD3, RGS12, IHO1, RIF1, SMC6, MKI67, TTN, CENPA, SMC5, SMC2, CENPC, RCC1, CHMP1A, KIFAP3, NIFK, CHAMP1
Mitochondrial Intermembrame Space	GO:0005758	57	COA4, PNPT1, PRELID2, PRELID1, AGK, TIMM29, TRAP1, HSD3B1, UQCC2, HSD3B2, SIRT5, NME4, TIMM23, HAX1, PINK1, ARL2BP, CYCS, NDUFS1, STOML2, DIABLO, NDUFB7, PANK2, CLPB, CPOX, TIMM10, PPOX, STMP1, MYOC, PRELID3A, COA7, PRELID3B, COA6, NDUFA8, TIMM8A, CMC4, CHCHD10, SOD1, GATM, TIMM10B, SDHAF3, STAR, FBXL4, THOP1, MICU2, MICU1, TIMM9, COX17, COX19, AK2, HTRA2, ARL2, TRIAP1, NLN, CHCHD4, BLOC1S1, OPA1, AIFM1, CHCHD2
Organelle Envelope Lumen	GO:0031970	63	COA4, PNPT1, PRELID2, PRELID1, AGK, TIMM29, TRAP1, HSD3B1, UQCC2, HSD3B2, TUBB, SIRT5, NME4, TIMM23, HAX1, CCAR1, PINK1, ARL2BP, CYCS, NDUFS1, APP, STOML2, DIABLO, NDUFB7, PANK2, CLPB, CPOX, TIMM10, PPOX, STMP1, ALOX5, MYOC, PRELID3A, COA7, PRELID3B,

			COA6, NDUFA8, TIMM8A, CMC4, CHCHD10, PLAAT1, SORL1, SOD1, GATM, TIMM10B, SDHAF3, STAR, FBXL4, THOP1, MICU2, MICU1, TIMM9, COX17, COX19, AK2, HTRA2, ARL2, TRIAP1, NLN, CHCHD4, BLOC1S1, OPA1, AIFM1, CHCHD2
Respiratory Chain Complex I	GO:0045271	41	NDUFA9, NDUFA8, NDUFA7, NDUFA6, NDUFA5, NDUFA4, NDUFA3, NDUFA2, NDUFA1, NDUFC2, NDUFC1, NDUFS8, NDUFS7, NDUFAB1, NDUFS6, NDUFS5, NDUFS4, NDUFS3, NDUFS2, NDUFAF1, NDUFS1, NDUFB9, NDUFB8, NDUFB7, NDUFB11, NDUFA12, NDUFB6, NDUFA13, NDUFA10, NDUFB5, NDUFB10, NDUFB4, NDUFA11, NDUFB3, NDUFB2, NDUFB1, NDUFC2-KCTD14, FOXRED1, WDR93, NDUFV3, NDUFV2, NDUFV1
Mitochondrial Respiratory Chain Complex I	GO:0005747	41	NDUFA9, NDUFA8, NDUFA7, NDUFA6, NDUFA5, NDUFA4, NDUFA3, NDUFA2, NDUFA1, NDUFC2, NDUFC1, NDUFS8, NDUFS7, NDUFAB1, NDUFS6, NDUFS5, NDUFS4, NDUFS3, NDUFS2, NDUFAF1, NDUFS1, NDUFB9, NDUFB8, NDUFB7, NDUFB11, NDUFA12, NDUFB6, NDUFA13, NDUFA10, NDUFB5, NDUFB10, NDUFB4, NDUFA11, NDUFB3, NDUFB2, NDUFB1, NDUFC2-KCTD14, FOXRED1, WDR93, NDUFV3, NDUFV2, NDUFV1
Intermediate Filament	GO:0005882	50	CSNK1A1, KRT5, KRT4, KRT3, KRT2, KRT14, KRT13, KRT8, KRT7, BFSP1, KRT10, VMAC, BFSP2, KRT18, KRT16, VIM, PKP2, NES, PLEC, PKP1, RTN2, FBF1, SLC1A4, EVPL, KRT20, PNN, FAM83H, NCKIPSD, GPER1, PRPH, KRT6C, DSP, KRT71, DST, KRT76, KRT75, CLIP1, EPPK1, HLA-DRB1, KRT82, FLG, MACF1, SYNM, PPL, EVPLL, KRT84, TCHP, MICAL1, TLK2, UPP2
Desmosome	GO:0030057	17	B4GALT1, CDSN, JUP, KLHL24, KAZN, DSG1, PKP2, DSG2, DSG3, PKP4, UBA1, DSG4, DSC1, DSC2, PKP1, JAM3, DSC3
CMG Complex	GO:0071162	10	GINS2, GINS3, GINS1, GINS4, MCM4, MCM5, MCM6, MCM7, MCM2, MCM3
Spindle Microtubule	GO:0005876	61	BOD1, EML3, CENPE, ZW10, FAM161A, KIF4A, PAFAH1B1, CDK1, CALM2, CALM3, CALM1, SKA1,

KIF11, HNRNPU, AURKA, CLTC, AURKC, SKA2, AURKB, SKA3, CSNK1D, CAPN6, CLASP1, CEP295, KNTC1, CLASP2, MAP9, PARP4, CCDC57, PLK1, HAUS5, HAUS4, PRC1, HAUS7, RMDN3, HAUS6, RMDN2, HAUS1, RMDN1, RAB11A, TUBG1, HAUS3, HAUS2, KIF18A, PSRC1, KIF18B, KIF2A, XIAP, BIRC5, BOD1L1, NUMA1, BOD1L2, ARL3, TTL, POLB, KIF3B, HAUS8, KIF3A, TOGARAM1, TOGARAM2, KIFAP3