Translational Oncology
www.transonc.com

Volume 11 Number 1 February 2018 pp. 168–174 168

Decreased plasma DEK Oncogene Levels Correlate with p16-Negative Disease and Advanced Tumor Stage in a Case–Control Study of Patients with Head and Neck Squamous Cell Carcinoma

Trisha Wise-Draper[*], Arun Sendilnathan[*], Sarah Palackdharry[*], Nicholas Pease[†], Julianne Qualtieri[‡], Randall Butler[‡], Nooshin Hashemi Sadraei[*], John C. Morris[*], Yash Patil[§], Keith Wilson[§], Jonathan Mark[§], Keith Casper[¶], Vinita Takiar[#], Adam Lane[**] and Lisa Privette Vinnedge[**]

*Division of Hematology-Oncology, Department of Internal Medicine, University of Cincinnati, Cincinnati, OH, 45267; †Department of Bioengineering, University of Washington, Seattle, WA 98105; ‡Department of Pathology, University of Cincinnati, Cincinnati, OH, 45267; §Department of Otolaryngology- Head and Neck Surgery, University of Cincinnati, Cincinnati, OH, 45267; ¶Department of Head and Neck Surgery, University of Michigan, Ann Arbor, MI 48109; #Department of Radiation Oncology, University of Cincinnati, Cincinnati, OH, 45267; **Cancer and Blood Diseases Institute, Cincinnati Children's Medical Center, Cincinnati, OH, 45229

Abstract

Head and neck cancer (HNC) remains the sixth most common malignancy worldwide and survival upon recurrence and/or metastasis remains poor. HNSCC has traditionally been associated with alcohol and nicotine use, but more recently the Human Papilloma Virus (HPV) has emerged as a favorable prognostic risk factor for oropharyngeal HNSCC. However, further stratification with additional biomarkers to predict patient outcome continues to be essential. One candidate biomarker is the DEK oncogenic protein, which was previously detected in the urine of patients with bladder cancer and is known to be secreted by immune cells such as macrophages. Here, we investigated if DEK could be detected in human plasma and if DEK levels correlated with clinical and pathological variables of HNSCC. Plasma was separated from the peripheral blood of newly diagnosed, untreated HNSCC patients or age-matched normal healthy controls and analyzed for DEK protein using ELISA. Plasma concentrations of DEK protein were lower in p16-negative tumors compared to both normal controls and patients with p16-positive tumors. Patients with lower plasma concentrations of DEK were also more likely to have late stage tumors and a lower white blood cell count. Contrary to previously published work demonstrating a poor prognosis with high intratumoral DEK levels, we show for the first time that decreased concentrations of DEK in patient plasma correlates with poor prognostic factors, including HPV-negative status as determined by negative p16 expression and advanced tumor stage.

Translational Oncology (2018) 11, 168–174

Introduction

Head and neck cancer (HNC) remains a global health concern accounting for 650,000 new cases each year, resulting in 350,000 deaths worldwide. The identification of human papillomavirus (HPV) infection as a favorable prognostic factor has fueled clinical studies investigating de-intensification strategies in this HPV positive disease subset. However, the overall survival of HPV

Address all correspondence to: Lisa Privette Vinnedge, Ph.D., Division of Oncology, Cancer and Blood Diseases Institute, Cincinnati Children's Hospital Medical Center, MLC 7018, 3333 Burnet Avenue, Cincinnati, OH 45229.
E-mail: Lisa.Privette@cchmc.org
Received 31 October 2017; Revised 1 December 2017; Accepted 4 December 2017

© 2017 The Authors. Published by Elsevier Inc. on behalf of Neoplasia Press, Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
1936-5233/18
https://doi.org/10.1016/j.tranon.2017.12.001

negative tumors as well as recurrent tumors remains poor despite intensive therapy. Early stage localized tumors are often cured with a single modality including surgical resection or radiation therapy; however, locally advanced HNC often requires a multi-modality approach. Despite primary intensive treatment of locally advanced disease, relapse free survival at 3 years remain at 30–50% in primary surgically resected disease [1]. Overall survival remains poor in the recurrent and metastatic setting despite aggressive treatment with median overall survival being 7–10.5 months [2]. Even though the addition of targeted agents such as the EGFR inhibitor, cetuximab, has resulted in an incremental rise in overall survival both in locally advanced and metastatic disease, the overall impact has been minimal [2–4].

Patients with HPV positive HNC often have an excellent prognosis and, if patients have less than a 10 pack-year smoking history, greater than 90% chance of cure in the locally advanced setting [5]. Importantly, HPV E7 induces expression of the human DEK oncogene [6]. DEK is primarily a chromatin structural and remodeling protein and has been shown to be an important driver of tumorigenesis *in vitro* and *in vivo* [7–13]. DEK over-expression in tumors, compared to adjacent normal tissue, has been observed in all cases of HNSCC tested to date and has also been observed with high frequency in breast cancer, melanoma, and many other solid tumors [8,14–16]. DEK over-expression induces cellular proliferation and invasion/metastasis both in cell culture and mouse models [13,17,18]. Furthermore, recent work has demonstrated that DEK over-expression is an independent prognostic factor predicting poor outcome in multiple solid tumors, including pancreatic ductal adenocarcinoma, gastric adenocarcinoma, breast cancer, melanoma, neuroendocrine prostate cancer, and non-small cell lung carcinoma [16,19–22].

Interestingly, despite largely being a chromatin-bound protein, DEK has been shown to be secreted by IL-8 treated macrophages *in vitro* and subsequently can function as a chemotactic factor for neutrophils, CD8+ T cells, and natural killer (NK) cells [23]. Extracellular DEK protein and auto-antibodies also have been detected in patients with several types of autoimmune diseases, including in the synovial fluid of patients with juvenile idiopathic arthritis [24–28]. Furthermore, extracellular DEK protein was also detected in the urine of bladder carcinoma patients [29]. Thus, we hypothesized that DEK would be present at different concentrations in HNC patient plasma compared to normal healthy controls. In this study, peripheral blood was collected from newly diagnosed, treatment naive HNSCC patients or age-matched normal healthy controls. Plasma was separated from the samples and subjected to DEK specific ELISA. Plasma DEK concentration levels were compared to normal controls, and to clinical and pathological variables. Interestingly, despite elevated intratumoral levels of DEK protein compared to healthy tissue, there was a trend to lower levels of DEK in HNC patient plasma compared to healthy controls in those patients that were HPV-negative, as determined by immunohistochemical staining for p16. Additionally, low DEK plasma levels correlate with advanced stage, and hence a likely poor prognosis, as well as a lower white blood cell count. Together our observations suggest plasma DEK levels correlate with important prognostic factors for HNSCC. Further characterization may aid in predicting patient outcome to various treatment modalities and may give insight to novel treatment strategies.

Methods

Patient Selection

Study participants included patients with a known or suspected diagnosis of HNSCC or normal healthy controls. Key inclusion criteria included the ability to understand and sign informed consent, collection of samples during routine procedures (except for normal healthy patients), and patients must have had adequate bone marrow and overall systemic function to withstand an extra blood draw. Key exclusion were patients with prior treatment, or any patients who did not meet the above and for healthy controls, patients with any history of cancer or auto-immune disease were excluded. Key demographics of the population tested are presented in Table 1. Median age of healthy controls ("NML") was 51.94 while median age of HNSCC patients was 56.67. Two HNSCC patients presented with multiple malignancies and were not included in analysis beyond detecting DEK concentrations. Not all control participants answered each question regarding demographics and lifestyle habits. Complete blood counts and tumor p16 status were obtained from chart review and were performed as part of standard care by the University of Cincinnati Department of Pathology and Laboratory Medicine. Written informed consent was obtained from all subjects. All patients and healthy controls were informed of the purpose of this research program and provided their written, informed consent. The study protocol was approved by the University of Cincinnati Institutional Review Board and is study protocol numbers 2014–6326 and 2014–4755.

Blood Collection and Analysis

Plasma was isolated from whole blood collected in EDTA tubes. Plasma was transferred to cryovials in 1 ml aliquots and stored at –80°C until used for analysis. Additional blood samples from patients were collected simultaneously and analyzed by University of Cincinnati Hospital for complete blood counts with differential, as part of standard care. Patient and control plasma was diluted 2-fold in sample buffer prior to analysis with a DEK-specific ELISA performed according to manufacturer's directions (Cusabio, Wuhan, China) and measured with a Molecular Diagnostics microplate reader and Softmax Pro 3.1 software.

Immunohistochemistry

Archived biopsy or resection human head and neck squamous cell carcinoma tissue were obtained with IRB approval. Tissues were fixed in formalin, embedded in paraffin, sectioned at 5 μm thickness, and fixed on to slides. Fixed paraffin sections were deparaffinized in xylene

Table 1. Demographics of Study Participants

Variable	Controls	HNC Patients	*P*
Race	Caucasian (n = 27)	Caucasian (n = 35)	0.0066
	Non-Caucasian (n = 10)	Non-Caucasian (n = 1)	
Sex	M (n = 10)	M (n = 30)	<0.0001
	F (n = 27)	F (n = 6)	
Diabetes Mellitus	Y (n = 6)	Y (n = 4)	0.7361
	N (n = 31)	N (n = 31)	
Auto-Immune	Y (n = 0)	Y (n = 2)	0.2328
	N (n = 37)	N (n = 33)	
Smoke	Any History (n = 17)	Any History (n = 28)	0.0119
	No History (n = 17)	No History (n = 7)	
Alcohol	Y (n = 15)	Y (n = 17)	0.6356
	N (n = 22)	N (n = 18)	

and incubated in sodium citrate solution for antigen retrieval. Sections were treated with the anti-mouse IgG Elite Vectastain ABC kit (Vector Labs, Burlingame, CA, USA) for detection of DEK (BD Biosciences) and Ki67 (ThermoScientific), which were diluted 1:500 and 1:1000, respectively. Sections were stained with Vector Laboratories 3,3′-diaminobenzidine, counterstained with hematoxylin and 0.2% ammonia hydroxide, and mounted with Permount (Fisher Scientific, Pittsburgh, PA, USA). Additional staining for p16 was performed using a mouse monoclonal antibody to p16 (Roche) on a Ventana Benchmark LT automated immunostainer according to standard protocol. Positive and negative controls were included routinely. Scoring criteria for p16 positive tumor status was ≥70% tumor cells staining positive. Ki67 scoring was determined as percent of tumor cells staining positive, described in increments of 10%. DEK scoring was determined by multiplying an intensity score (0/negative, 1/weak, 2/moderate, 3/strong) by the distribution score (0/no staining, 1/<33% positive, 2/33–66% positive, 3/>66% positive) to achieve a total score from a range between 0–9.

Statistics

Descriptive statistics were reported for continuous and categorical variables as median (range), and frequency, respectively. DEK levels were compared across groups with Wilcoxon rank sum tests. Fisher exact tests were used to compare two categorical variables. Correlation was found using Pearson's method, except in the instance of comparing histological staining for Ki67 and DEK, in which case Spearman correlation (ρ) was used. To adjust for p16 status, a multiple linear regression model was used. All computations were done in R version 3.2.4 (Vienna, Austria). All comparisons were two-sided, statistical significance was set at .05.

Results

Common Patient Risk Factors and Characteristics Do Not Influence DEK Plasma Protein Levels

Between December 2014 and December 2015, 38 newly diagnosed HNSCC patients and 37 age-matched normal healthy control patients were consented for blood collection. Comparing control and HNSCC patients, there were significant differences in sex and race distribution and smoking status, all of which are known risk factors for HNSCC (Table 1), thus, our study participants are representative of the HNSCC patient population [30]. Blood was collected prior to treatment, processed, and plasma was separated for analysis. Using DEK specific ELISA, DEK protein was detected in both normal healthy control and HNSCC patients. There was not a significant difference between plasma DEK concentrations in healthy controls and the collective HNSCC patient population (Figure 1*A*). Many other common patient characteristics and risk factors are known to be associated with HNSCC as well as prognosis. Therefore, DEK plasma levels were compared with these confounding factors such as smoking status, alcohol use, race and sex. DEK plasma levels did not correlate with any of these common clinical and pathological variables tested in either normal healthy controls or HNSCC patients (Table 2).

Plasma DEK Levels Correlate with HPV Status in HNSCC Patients

Although we noticed no difference in plasma DEK levels comparing normal and HNSCC patients, we did observe a larger range of values in patient samples. Importantly, HNSCC comprises a heterogenous group of cancers. Therefore, patient samples were separated into clinical anatomical sites. Patients with oropharyngeal tumors had statistically significant higher levels of DEK plasma protein compared to laryngeal carcinomas (Figure 1*B*).

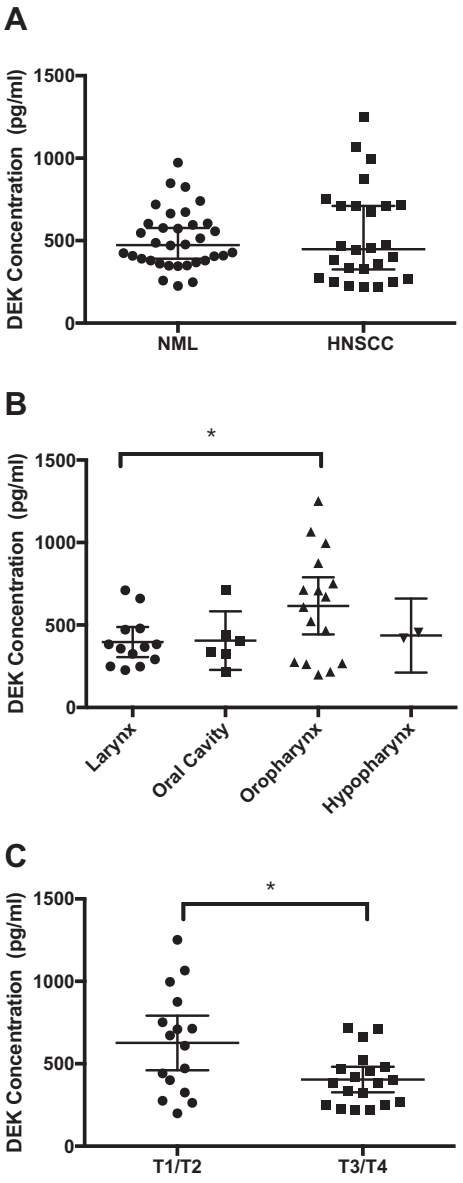

Figure 1. Plasma DEK concentrations are higher in cancers of the oropharynx compared to other sites of head and neck cancers. (A) Plasma DEK levels were measured by ELISA in healthy (NML) samples (median 409.5 pg/ml) and in patients with head and neck squamous cell carcinomas (HNSCC, median 473.2 pg/ml). No differences were detected between the medians. (B) Plasma DEK concentrations among various tissues of origin within HNSCC patients. Patients with cancers in the oropharynx had higher plasma DEK concentrations (616 ± 81.25 pg/ml, N = 16) compared to other sites, including larynx (396.4 ± 41.82 pg/ml, N = 13) as determined by ELISA assay. (C) Plasma DEK concentrations were compared between patients with small tumors (T1/T2) (median 671.5 pg/ml; range 199.2–1252.1 pg/ml, N = 15) or large tumors (T3/T4) (median 401.0 pg/ml; range 227.0–714.5 pg/ml, N = 16) as determined when calculating clinical TNM stage. Patients with larger tumors, and thus advanced stage, had lower DEK levels. (P = .0191). Median and 95% confidence intervals are shown. * P <.05.

Table 2. Clinical and Pathological Characteristics of Study Participants and Plasma DEK Levels by Group

	Controls			HNC Patients		
Variable		DEK Mean (pg/ml) (Range)	p-Value*		DEK Mean (pg/ml) (Range)	*P* Value*
Race	Caucasian (n = 27)	476.33 (226.1–848.6)	0.9063	Caucasian (n = 35)		
	Non-Caucasian (n = 10)	416.3 (345.7–2192.4)		Non-Caucasian (n = 1)	No comparison possible	
Sex	M (n = 10)	400.5 (248.6–719.3)	0.2424	M (n = 30)	409.5 (172.2–1252.2)	0.4935
	F (n = 27)	476.3 (226.1–2192.1)		F (n = 6)	566.4 (249.0–714.5)	
Diabetes Mellitus	Y (n = 6)	671.9 (408.2–2192.4)	0.0391	Y (n = 4)	384.2 (262.7–714.5)	0.7834
	N (n = 31)	429.7 (226.1–848.6)		N (n = 31)	454.1 (199.2–1252.1)	
Auto-Immune	Y (n = 0)			Y (n = 2)	629.15 (382.71–875.6)	0.5244
	N (n = 37)	No comparison possible		N (n = 33)	442.5 (199.2–1252.1)	
Smoke	Any History (n = 17)	440 (226.1–2192.4)	0.5144	Any History (n = 28)	713.22 (199.2–775.6)	0.2294
	No History (n = 17)	473.3 (347.5–973.2)		No History (n = 7)	409.5 (217.2–1252.1)	
Alcohol	Y (n = 15)	429.7 (248.6–2192.4)	0.7025	Y (n = 17)	454.1 (217.2–1065.3)	0.5034
	N (n = 22)	530.5 (226.1–973.2)		N (n = 18)	430.7 (199.2–1252.1)	

Oropharyngeal tumors are often HPV positive, which carry a favorable prognosis, and HPV E7 protein has previously been shown to up-regulate *DEK* expression [6,31]. Given the latter, as well as that HPV positive tumors have been found to harbor different mutations based on TCGA analyses [32], samples were stratified for HPV status using p16 immunohistochemistry stain as a surrogate marker. As expected, HNSCC patients with p16 positive, and therefore likely HPV-positive, disease had the highest levels of DEK protein in their plasma (668.6 pg/ml) which was significantly higher compared to HNSCC patients with p16-negative disease ($P = .0062$). Furthermore, plasma DEK protein levels were lowest in patients with p16 negative HNSCC (390.4 pg/ml), and were significantly lower than concentrations found in controls (473.2 pg/ml; $P = .009$) (Table 3). These findings were similar whether p16 status was compared in oropharyngeal tumors or all sites (data not shown). Together, DEK plasma concentrations do not differ, on average, between all HNC patients and healthy controls; however, upon stratification based on p16 status, there is a statistically significant difference between all three groups (Table 3).

DEK Plasma Levels Decrease with Larger Tumor Size

Advanced stage in HNSCC, especially advanced T stage, is correlated with worse patient overall survival. When samples were analyzed by tumor stage, plasma DEK concentrations were significantly lower in patients with T3 and T4 stage disease compared to early stages T1 or T2 (Figure 1*C*). Given that DEK levels are decreased in HPV/p16-negative patients and in patients with advanced disease, low plasma DEK concentrations appear to correlate with variables associated with poor disease outcomes. Due to this difference in tumor T stage and DEK concentrations, we next assessed patient survival. We did not detect a difference in survival based on DEK plasma concentrations ($P = .42$); however, analysis comparing plasma DEK levels with treatment response is limited here by a small sample size and short time for follow-up. We were able to confirm in our cohort that HPV negative HNSCC has a poor clinical outcome compared to HPV positive disease ($P = .0012$, data not shown).

Table 3. Plasma DEK Levels Correlate with p16 Status

All Patients			
	Variable	DEK Mean (Range)	*P* Value*
Group	HNC (n = 36)	430.7 (172.2–1252.1)	0.3461
	Control (n = 38)	473.2 (226.1–2192.1)	
p16 status	Control (n = 38)	473.2 (226.1–2192.1)	
	HNC		
	p16 (n = 14)	668.6 (199.2–1252.1)	
	No p16 (n = 22)	390.4 (172.2–714.5)	
		Control *vs.* p16+	0.0433
		Control *vs.* p16-	0.0094
		p16+ *vs.* p16-	0.0062

Table 4. Correlation of Plasma DEK Concentrations with Complete Blood Cell Counts

		Univariate			Adjusted for p16 Status	
Cells	Median (Range)	Correlation	Coefficient	*P* Value	Coefficient	*P* Value
WBC	7.8 (4.5–18.3)	0.26	21.2	0.15	29.1	0.029
RBC	4.7 (3.1–5.7)	0.13	49.5	0.49	0.28	0.997
Platelet	239 (112–653)	0.37	0.82	0.04	0.64	0.095
ANC	5255 (2214–8749)	0.13	0.02	0.53	0.03	0.231
ALC	1630 (233–3510)	0.43	0.12	0.03	0.10	0.057
AMC	675 (50–1245)	0.14	0.12	0.49	0.17	0.282
Eosino	144 (0–676)	0.50	0.77	0.01	0.66	0.019
Basophil	49.6 (12–131)	0.27	2.22	0.19	3.10	0.482

WBC: white blood cell; RBC: red blood cell; ANC: absolute neutrophil count; ALC: absolute lymphocyte count; AMC: absolute monocyte count; Eosino: eosinophil count. Correlation determined by linear regression.

Low DEK Plasma Levels Correlate with Decreased Pre-Treatment White Blood Cell Counts

In vitro studies have shown that DEK can be a chemoattractant for neutrophils, cytotoxic CD8+ T cells, and NK cells, and is released by macrophages treated with the pro-inflammatory cytokine IL-8 [23]. Furthermore, elevated extracellular levels of DEK are observed in biofluids collected locally from affected tissues in patients with inflammatory autoimmune diseases (*i.e.* synovial fluid from patients with juvenile idiopathic arthritis) [24,27]. This suggests that extracellular DEK may also be associated with differences in tumor immune responses. Indeed, after adjustment for p16 status, patients with low DEK levels correlated with a lower white blood cell (WBC) count compared to patients with higher DEK plasma concentrations, with lymphocytes (ALC) and eosinophils comprising the majority of this difference (Figure 3 and Table 4). Interestingly, there was also a direct correlation between plasma DEK concentrations and platelet counts and a trend towards a direct correlation with basophil counts (Table 4).

Intratumoral versus Plasma DEK Levels

Previously published work has found a significant positive correlation between intratumoral DEK and p16 expression by immunohistochemistry [33]. We next analyzed intratumoral DEK expression by immunohistochemistry and compared it to other histological markers of disease and plasma DEK concentrations. We did not observe a difference between intratumoral DEK immunohistochemical staining and p16 status; which may be due to the small sample size tested here. There also was no correlation between intratumoral DEK staining scores and plasma DEK concentrations ($\rho = 0.22$, $P = .31$, N = 25), suggesting that the tumor cells may not be the source of DEK protein detected in plasma. However, we did observe a strong positive correlation between intratumoral DEK immunohistochemistry score and the percentage of tumor cells staining positive for proliferation marker Ki67 (Figure 2), a finding which replicates previous work associating DEK with cell proliferation (Spearman correlation $\rho = 0.77$, $P = .0098$, N = 10) [15,17,18,20,34].

Discussion

Previous work has demonstrated that DEK could be detected in the urine of bladder cancer patients and, thus, may be useful as a disease biomarker [29]. Given this, and that activated macrophages can secrete DEK, we hypothesized that DEK may be measured in patient plasma and used as a biomarker for other solid tumors. We are the first to report that, indeed, DEK can be detected in human plasma at significant concentrations ranging from 172–2192 pg/ml. Although DEK levels were not predictive of the presence of HNSCC in general, further analysis demonstrated that DEK concentrations correlated with several important pathological characteristics and prognostic factors of HNSCC tumors, including tumor site, HPV status as determined by p16 staining, and tumor size. Although we did not find an association with patient outcome, our study was limited by a small patient size and a short time-to-follow-up of only 1–2 years. Additional studies with larger sample sizes and longer follow-up times are warranted.

Future work will be needed to determine why p16+ cancers correlate with increased plasma DEK levels. Several possibilities exist to potentially explain this phenomenon. First, given the role of extracellular DEK in inflammatory and anti-pathogenic immune responses such as neutrophil extracellular traps [42], elevated DEK plasma concentrations may be due to an immune response to the HPV antigens associated with p16+ disease. Alternatively, it may be that excess DEK protein produced as a result of the HPV E7 oncoprotein [6,31] is secreted by the HPV-transformed cancer cells. This hypothesis is not likely, as we did not observe a correlation between plasma and intratumoral levels of DEK. Finally, work *in*

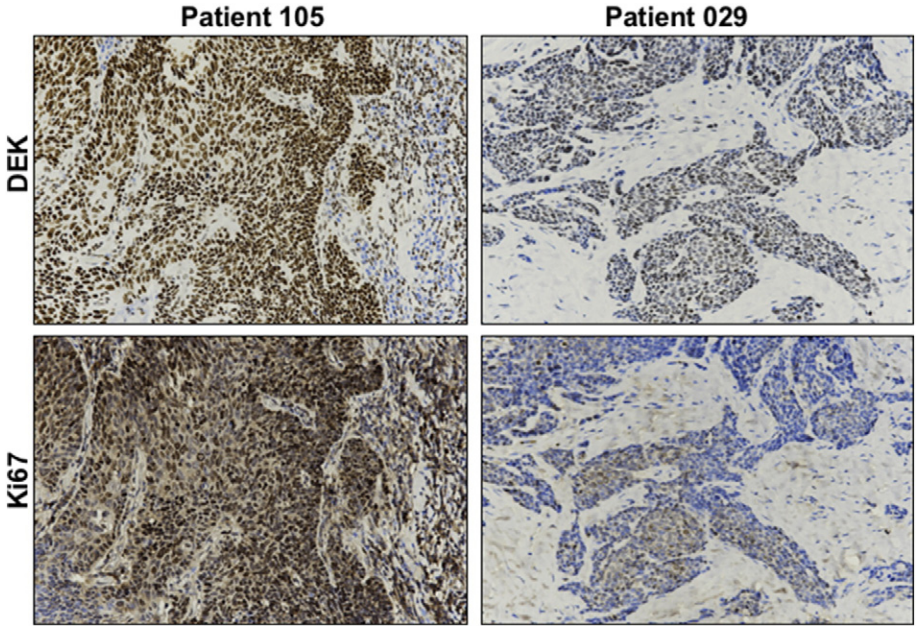

Figure 2. Intratumoral levels of DEK correlate with Ki67 staining as a marker of cancer cell proliferation. Representative examples of patient samples demonstrating the positive correlation of intratumoral DEK and Ki67 protein levels by immunohistochemistry. The patient on the left demonstrates a tumor with both high DEK and Ki67 staining while the patient on the right is an example of low DEK and Ki67 staining (Spearman correlation $\rho = 0.77$, $p = .0098$, N = 10).

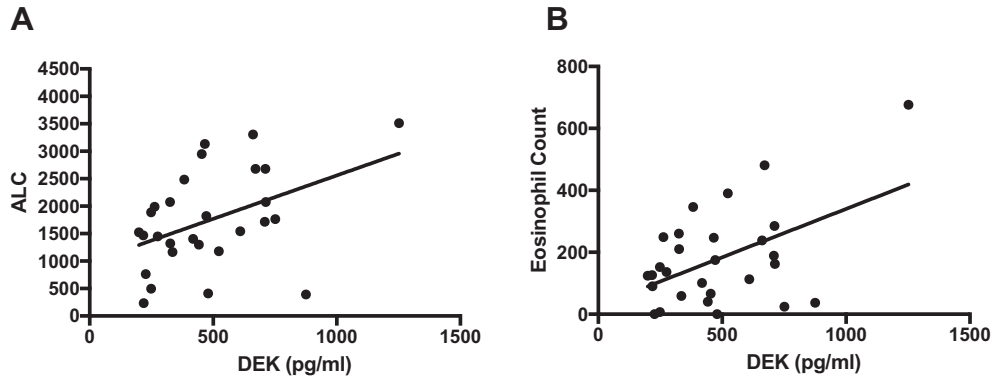

Figure 3. Plasma DEK concentrations positively correlate with blood counts. Univariate linear regression of (A) absolute lymphocyte counts (ALC) and (B) eosinophil counts as a function of plasma DEK concentrations in pg/ml. Correlation and *P* values are shown in Table 4.

vitro has demonstrated that DEK is poly(ADP-ribosyl)ated and released into the extracellular space by apoptotic cells. Therefore, apoptotic cancer cells may explain the increased plasma levels of DEK and its association with favorable prognostic markers.

Interestingly, we observed a direct correlation between plasma DEK concentrations and white blood cell counts, largely due to differences in lymphocytes and eosinophils. Importantly, this was independent of the p16 status, as a marker of HPV infection, of the tumors. Exogenous recombinant human DEK protein has been shown to suppress the proliferation of hematopoietic progenitor cells isolated from human cord blood *in vitro*, which could have an impact on hematopoiesis *in vivo* [35]. Thus, secreted DEK levels may be directly influencing hematopoiesis in patients, resulting in different CBC results. However, in our studies we observe a positive correlation between plasma DEK levels and elevated blood cell counts, whereas *in vitro* studies demonstrated that exogenous DEK was inhibitory to hematopoiesis. This suggests that the observations reported here, directly correlating DEK plasma levels and CBC results, are likely not due to the influence of DEK on hematopoiesis. We hypothesize that this correlation may be indicative of an anti-tumor immune response. Tumor associated eosinophils recently were shown to facilitate the anti-tumor immune response by enhancing CD8+ T cell infiltration and have been linked to favorable prognosis in oral squamous cell carcinomas [36,37]. Several studies analyzing absolute lymphocyte count (ALC) in pre-operative patients with solid tumors have demonstrated that lower lymphocyte counts, as we observed with low DEK plasma concentrations, are associated with decreased survival rates [38–41]. Additional studies are needed in order to determine if the lower eosinophil and lymphocyte counts on CBC from patients with low plasma DEK concentrations correlate with limited intratumoral immune responses and poor survival.

The finding that low plasma DEK concentrations correlate with factors associated with poor outcome is the opposite of numerous reports demonstrating that high intratumoral staining for DEK is an independent prognostic factor of poor outcome. However, this is not entirely surprising, given the previous reports linking extracellular DEK protein with activation of the immune response. Thus, we hypothesize that extracellular DEK protein, as found in patient plasma, may be an indicator of an inflammatory, anti-tumor immune response and, thus, is temporarily beneficial to limit tumorigenesis and disease progression. Therefore, future efforts to therapeutically target DEK in malignancies should be approached with caution as it may negatively impact the anti-tumor immune response.

Conclusions

Low plasma DEK concentrations were associated with p16-negative disease, which is typically found in sites other than the oropharynx, and has a poor prognosis. In addition, low plasma DEK levels correlated with larger tumor size, a measurement used during TNM staging. Finally, we also noted a direct correlation between plasma DEK levels and pre-operative ALC. Combined, the results indicate that lower amounts of DEK in patient plasma may be predictive of advanced disease status and poor treatment outcomes. Importantly, these findings should also be taken into consideration when developing DEK-targeting therapeutics. Further studies are needed to investigate the prognostic value of plasma DEK levels and the interplay between extracellular DEK in patient biofluids and the anti-tumor immune response.

Acknowledgements

Trisha Wise-Draper, MD, PhD, was supported during this work by the Clinical Scientist Training Program at the University of Cincinnati and currently supported by a CTSA KL2 mentored training grant and internal HOTSA pilot grant from the University of Cincinnati. Lisa Privette Vinnedge, PhD was supported by pilot grant funds from the University of Cincinnati Center for Clinical and Translational Science and Training and the Breast Cancer Pilot Grant Program from the Marlene Harris Ride Cincinnati Foundation.

References

[1] Oksuz DC, Prestwich RJ, Carey B, Wilson S, Senocak MS, Choudhury A, Dyker K, Coyle C, and Sen M (2011). Recurrence patterns of locally advanced head and neck squamous cell carcinoma after 3D conformal (chemo)-radiotherapy. *Radiat Oncol* **6**, 54. https://doi.org/10.1186/1748-717X-6-54.

[2] Vermorken JB, Mesia R, Rivera F, Remenar E, Kawecki A, Rottey S, Erfan J, Zabolotnyy D, Kienzer HR, and Cupissol D, et al (2008). Platinum-based chemotherapy plus cetuximab in head and neck cancer. *N Engl J Med* **359**(11), 1116–1127. https://doi.org/10.1056/NEJMoa0802656.

[3] Ang KK, Zhang Q, Rosenthal DI, Nguyen-Tan PF, Sherman EJ, Weber RS, Galvin JM, Bonner JA, Harris J, and El-Naggar AK, et al (2014). Randomized phase III trial of concurrent accelerated radiation plus cisplatin with or without cetuximab for stage III to IV head and neck carcinoma: RTOG 0522. *J Clin Oncol* **32**(27), 2940–2950. https://doi.org/10.1200/JCO.2013.53.5633.

[4] Bonner JA, Harari PM, Giralt J, Cohen RB, Jones CU, Sur RK, Raben D, Baselga J, Spencer SA, and Zhu J, et al (2010). Radiotherapy plus cetuximab for locoregionally advanced head and neck cancer: 5-year survival data from a phase 3 randomised trial, and relation between cetuximab-induced rash and survival. *Lancet Oncol* **11**(1), 21–28. https://doi.org/10.1016/S1470-2045(09)70311-0.

[5] Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tan PF, Westra WH, Chung CH, Jordan RC, and Lu C, et al (2010). Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med* **363**(1), 24–35. https://doi.org/10.1056/NEJMoa0912217.

[6] Wise-Draper TM, Allen HV, Thobe MN, Jones EE, Habash KB, Munger K, and Wells SI (2005). The human DEK proto-oncogene is a senescence inhibitor and an upregulated target of high-risk human papillomavirus E7. *J Virol* **79**(22), 14309–14317. https://doi.org/10.1128/JVI.79.22.14309-14317.2005.

[7] Alexiadis V, Waldmann T, Andersen J, Mann M, Knippers R, and Gruss C (2000). The protein encoded by the proto-oncogene DEK changes the topology of chromatin and reduces the efficiency of DNA replication in a chromatin-specific manner. *Genes Dev* **14**(11), 1308–1312.

[8] Adams AK, Hallenbeck GE, Casper KA, Patil YJ, Wilson KM, Kimple RJ, Lambert PF, Witte DP, Xiao W, and Gillison ML, et al (2014). DEK promotes HPV-positive and -negative head and neck cancer cell proliferation. *Oncogene* **0**, 1. https://doi.org/10.1038/onc.2014.15.

[9] Kappes F, Waldmann T, Mathew V, Yu J, Zhang L, Khodadoust MS, Chinnaiyan AM, Luger K, Erhardt S, and Schneider R, et al (2011). The DEK oncoprotein is a Su(var) that is essential to heterochromatin integrity. *Genes Dev* **25**(7), 673–678.

[10] Privette Vinnedge LM, Kappes F, Nassar N, and Wells SI (2013). Stacking the DEK: from chromatin topology to cancer stem cells. *Cell Cycle* **12**(1), 51–66. https://doi.org/10.4161/cc.23121.

[11] Ivanauskiene K, Delbarre E, McGhie JD, Kuntziger T, Wong LH, and Collas P (2014). The PML-associated protein DEK regulates the balance of H3.3 loading on chromatin and is important for telomere integrity. *Genome Res.* https://doi.org/10.1101/gr.173831.114.

[12] Wise-Draper TM, Mintz-Cole RA, Morris TA, Simpson DS, Wikenheiser-Brokamp KA, Currier MA, Cripe TP, Grosveld GC, and Wells SI (2009). Overexpression of the cellular DEK protein promotes epithelial transformation in vitro and in vivo. *Cancer Res* **69**(5), 1792–1799. https://doi.org/10.1158/0008-5472.CAN-08-2304.

[13] Shibata T, Kokubu A, Miyamoto M, Hosoda F, Gotoh M, Tsuta K, Asamura H, Matsuno Y, Kondo T, and Imoto I, et al (2010). DEK oncoprotein regulates transcriptional modifiers and sustains tumor initiation activity in high-grade neuroendocrine carcinoma of the lung. *Oncogene* **29**(33), 4671–4681.

[14] Khodadoust MS, Verhaegen M, Kappes F, Riveiro-Falkenbach E, Cigudosa JC, Kim DS, Chinnaiyan AM, Markovitz DM, and Soengas MS (2009). Melanoma proliferation and chemoresistance controlled by the DEK oncogene. *Cancer Res* **69**(16), 6405–6413.

[15] Liu S, Wang X, Sun F, Kong J, Li Z, and Lin Z (2012). DEK overexpression is correlated with the clinical features of breast cancer. *Pathol Int* **62**(3), 176–181. https://doi.org/10.1111/j.1440-1827.2011.02775.x.

[16] Ying G and Wu Y (2015). DEK: A novel early screening and prognostic marker for breast cancer. *Mol Med Rep* **12**(5), 7491–7495. https://doi.org/10.3892/mmr.2015.4380.

[17] Privette Vinnedge LM, Benight NM, Wagh PK, Pease NA, Nashu MA, Serrano-Lopez J, Adams AK, Cancelas JA, Waltz SE, and Wells SI (2015). The DEK oncogene promotes cellular proliferation through paracrine Wnt signaling in Ron receptor-positive breast cancers. *Oncogene* **34**(18), 2325–2336. https://doi.org/10.1038/onc.2014.173.

[18] Privette Vinnedge LM, McClaine R, Wagh PK, Wikenheiser-Brokamp KA, Waltz SE, and Wells SI (2011). The human DEK oncogene stimulates beta-catenin signaling, invasion and mammosphere formation in breast cancer. *Oncogene* **30**(24), 2741–2752. https://doi.org/10.1038/onc.2011.2.

[19] Sun J, Bi F, Yang Y, Zhang Y, Jin A, Li J, and Lin Z (2017). DEK protein overexpression predicts poor prognosis in pancreatic ductal adenocarcinoma. *Oncol Rep* **37**(2), 857–864. https://doi.org/10.3892/or.2016.5302.

[20] Ou Y, Xia R, Kong F, Zhang X, Yu S, Jiang L, Zheng L, and Lin L (2016). Overexpression of DEK is an indicator of poor prognosis in patients with gastric adenocarcinoma. *Oncol Lett* **11**(3), 1823–1828. https://doi.org/10.3892/ol.2016.4147.

[21] Liu X, Qi D, Qi J, Mao Z, Li X, Zhang J, Li J, and Gao W (2016). Significance of DEK overexpression for the prognostic evaluation of non-small cell lung carcinoma. *Oncol Rep* **35**(1), 155–162. https://doi.org/10.3892/or.2015.4365.

[22] Wang X, Lin L, Ren X, Lin Z, Li Z, Li C, and Jin T (2014). High expression of oncoprotein DEK predicts poor prognosis of small cell lung cancer. *Int J Clin Exp Pathol* **7**(8), 5016–5023.

[23] Mor-Vaknin N, Punturieri A, Sitwala K, Faulkner N, Legendre M, Khodadoust MS, Kappes F, Ruth JH, Koch A, and Glass D, et al (2006). The DEK nuclear autoantigen is a secreted chemotactic factor. *Mol Cell Biol* **26**(24), 9484–9496.

[24] Mor-Vaknin N, Kappes F, Dick AE, Legendre M, Damoc C, Teitz-Tennenbaum S, Kwok R, Ferrando-May E, Adams BS, and Markovitz DM (2011). DEK in the synovium of patients with juvenile idiopathic arthritis: characterization of DEK antibodies and posttranslational modification of the DEK autoantigen. *Arthritis Rheum* **63**(2), 556–567. https://doi.org/10.1002/art.30138.

[25] Dong X, Michelis MA, Wang J, Bose R, DeLange T, and Reeves WH (1998). Autoantibodies to DEK oncoprotein in a patient with systemic lupus erythematosus and sarcoidosis. *Arthritis Rheum* **41**(8), 1505–1510.

[26] Dong X, Wang J, Kabir FN, Shaw M, Reed AM, Stein L, Andrade LE, Trevisani VF, Miller ML, and Fujii T, et al (2000). Autoantibodies to DEK oncoprotein in human inflammatory disease. *Arthritis Rheum* **43**(1), 85–93.

[27] Pease NA, Wise-Draper T, and Privette Vinnedge L (2015). Dissecting the Potential Interplay of DEK Functions in Inflammation and Cancer. *J Oncol* **2015**, 106517. https://doi.org/10.1155/2015/106517.

[28] Wichmann I, Respaldiza N, Garcia-Lozano JR, Montes M, Sanchez-Roman J, and Nunez-Roldan A (2000). Autoantibodies to DEK oncoprotein in systemic lupus erythematosus (SLE). *Clin Exp Immunol* **119**(3), 530–532.

[29] Datta A, Adelson ME, Mogilevkin Y, Mordechai E, Sidi AA, and Trama JP (2011). Oncoprotein DEK as a tissue and urinary biomarker for bladder cancer. *BMC Cancer* **11**, 234. https://doi.org/10.1186/1471-2407-11-234.

[30] Marur S and Forastiere AA (2016). Head and Neck Squamous Cell Carcinoma: Update on Epidemiology, Diagnosis, and Treatment. *Mayo Clin Proc* **91**(3), 386–396. https://doi.org/10.1016/j.mayocp.2015.12.017.

[31] Wise-Draper TM and Wells SI (2008). Papillomavirus E6 and E7 proteins and their cellular targets. *Front Biosci* **13**, 1003–1017.

[32] Gross AM, Orosco RK, Shen JP, Egloff AM, Carter H, Hofree M, Choueiri M, Coffey CS, Lippman SM, and Hayes DN, et al (2014). Multi-tiered genomic analysis of head and neck cancer ties TP53 mutation to 3p loss. *Nat Genet* **46**(9), 939–943. https://doi.org/10.1038/ng.3051.

[33] Smith EA, Kumar B, Komurov K, Smith SM, Brown NV, Zhao S, Kumar P, Teknos TN, and Wells SI (2017). DEK associates with tumor stage and outcome in HPV16 positive oropharyngeal squamous cell carcinoma. *Oncotarget* **8**(14), 23414–23426. https://doi.org/10.18632/oncotarget.15582.

[34] Lin L, Piao J, Ma Y, Jin T, Quan C, Kong J, Li Y, and Lin Z (2014). Mechanisms underlying cancer growth and apoptosis by DEK overexpression in colorectal cancer. *PLoS One* **9**(10)e111260. https://doi.org/10.1371/journal.pone.0111260.

[35] Broxmeyer HE, Kappes F, Mor-Vaknin N, Legendre M, Kinzfogl J, Cooper S, Hangoc G, and Markovitz DM (2011). DEK Regulates Hematopoietic Stem Engraftment and Progenitor Cell Proliferation. *Stem Cells Dev.* https://doi.org/10.1089/scd.2011.0451.

[36] Dorta RG, Landman G, Kowalski LP, Lauris JR, Latorre MR, and Oliveira DT (2002). Tumour-associated tissue eosinophilia as a prognostic factor in oral squamous cell carcinomas. *Histopathology* **41**(2), 152–157.

[37] Carretero R, Sektioglu IM, Garbi N, Salgado OC, Beckhove P, and Hammerling GJ (2015). Eosinophils orchestrate cancer rejection by normalizing tumor vessels and enhancing infiltration of CD8(+) T cells. *Nat Immunol* **16**(6), 609–617. https://doi.org/10.1038/ni.3159.

[38] Rochet NM, Kottschade LA, Grotz TE, Porrata LF, and Markovic SN (2015). The prognostic role of the preoperative absolute lymphocyte count and absolute monocyte count in patients with resected advanced melanoma. *Am J Clin Oncol* **38**(3), 252–258. https://doi.org/10.1097/COC.0b013e31829b5605.

[39] Li X, Han Z, Cheng Z, Yu J, Yu X, and Liang P (2014). Prognostic value of preoperative absolute lymphocyte count in recurrent hepatocellular carcinoma following thermal ablation: a retrospective analysis. *Onco Targets Ther* **7**, 1829–1835. https://doi.org/10.2147/OTT.S69227.

[40] Mehrazin R, Uzzo RG, Kutikov A, Ruth K, Tomaszewski JJ, Dulaimi E, Ginzburg S, Abbosh PH, Ito T, and Corcoran AT, et al (2015). Lymphopenia is an independent predictor of inferior outcome in papillary renal cell carcinoma. *Urol Oncol* **33**(9), 388.e19-25. https://doi.org/10.1016/j.urolonc.2014.06.004.

[41] Zhang Y, Ma C, Wang M, Hou H, Cui L, Jiang C, Sun J, and Qu X (2017). Prognostic significance of immune cells in the tumor microenvironment and peripheral blood of gallbladder carcinoma patients. *Clin Transl Oncol.* **19**(4), 477–488.

[42] Mor-Vaknin N, Saha A, Legendre M, Carmona-Rivera C, Amin MA, Rabquer BJ, Gonzales-Hernandez MJ, Jorns J, Mohan S, and Yalavarthi S, et al (2017). DEK-targeting DNA aptamers as therapeutics for inflammatory arthritis. *Nat Commun.* **8**, 14252. https://doi.org/10.1038/ncomms14252.