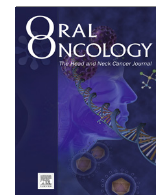
Oral Oncology 64 (2017) 65–72

Contents lists available at ScienceDirect

Oral Oncology

journal homepage: www.elsevier.com/locate/oraloncology



Defining the boundaries and expanding the utility of head and neck cancer patient derived xenografts

Adam D. Swick [a,1], Andrew P. Stein [a,1], Timothy M. McCulloch [d,e], Gregory K. Hartig [d,e], Irene M. Ong [b,e], Emmanuel Sampene [b], Prashanth J. Prabakaran [a], Cheng Z. Liu [c], Randall J. Kimple [a,e,*]

[a] Department of Human Oncology, University of Wisconsin School of Medicine and Public Health, Madison, WI 53705, USA
[b] Department of Biostatistics and Medical Informatics, University of Wisconsin School of Medicine and Public Health, Madison, WI 53705, USA
[c] Department of Pathology, University of Wisconsin School of Medicine and Public Health, Madison, WI 53705, USA
[d] Department of Otolaryngology, University of Wisconsin School of Medicine and Public Health, Madison, WI 53705, USA
[e] University of Wisconsin Carbone Cancer Center, University of Wisconsin School of Medicine and Public Health, Madison, WI 53705, USA

ARTICLE INFO

Article history:
Received 7 August 2016
Received in revised form 11 October 2016
Accepted 27 November 2016

Keywords:
Head and neck cancer
HNSCC
Patient derived xenograft
PDX
Mouse model

ABSTRACT

Background: Patient derived xenografts (PDXs) represent an essential tool in oncologic research, and we sought to further expand our repertoire of head and neck squamous cell carcinoma (HNSCC) while determining potential boundaries for this system.
Methods: We consented new patients for PDX development and determined if a 24-h time delay from tumor excision to xenograft implantation affected PDX establishment. We developed a tissue microarray (TMA) from formalin fixed, paraffin embedded PDXs and their subsequent passages and carried out quantitative immunohistochemistry for EGFR, pEGFR, pAkt, pERK and ERCC1. First and last passaged PDXs were compared via a paired *t*-test to examine for the stability of protein expression across passages. We performed a similar comparison of the mutational profile of the patient tumor and resulting xenografts using a targeted sequencing approach.
Results: No patient/tumor characteristics influenced PDX take rate and the 24-h time delay from tumor excision to xenograft implantation did not affect PDX establishment, growth or histology. There was no significant difference in biomarker expression between the first and last passaged PDXs for EGFR, pEGFR, pAkt, and ERCC1. For pERK there was a significant difference ($p = 0.002$), but further analysis demonstrated this only arose in three of 15 PDXs. Targeted sequencing revealed striking stability of passenger and likely driver mutations from patient to xenograft.
Conclusions: The stability of protein expression across PDX passages will hopefully allow greater investigation of predictive biomarkers in order to identify ones for further pre-clinical and clinical investigation.

© 2016 Elsevier Ltd. All rights reserved.

Introduction

Patient derived xenografts (PDXs), which are generated by directly implanting patient tumor tissue from a surgical resection or clinic biopsy either orthotopically or heterotopically into immunodeficient mice [1], have become a widely used model system for oncologic research. PDXs are hypothesized to more closely resemble a patient's primary tumor than cell lines, and their histologic [2,3] and molecular features [1,2,4,5] mirror those of the primary cancer. PDXs have demonstrated establishment rates between 30 and 80% of the time across a range of tumor types [1,2,4,6]. Following successful growth in the initial cohort of mice, tumors are excised and passaged into a new round of mice. In this manner, PDX tissue can be amplified and implanted into numerous mice to carry out therapeutic studies [6,7]. Additionally, molecular analyses can be performed on pre-treatment tumors to identify biomarkers related to therapeutic response (predictive biomarkers). This represents an important facet and usage for PDXs, especially for cancers such as head and neck squamous cell carcinoma (HNSCC) where no currently validated predictive biomarkers exist [8].

PDXs have become an essential tool in the preclinical development of novel therapeutics, with large cohort studies able to

* Corresponding author at: Department of Human Oncology, University of Wisconsin Comprehensive Cancer Center, 3107 WIMR, 1111 Highland Avenue, Madison, WI 53792, USA.
E-mail address: rkimple@humonc.wisc.edu (R.J. Kimple).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.oraloncology.2016.11.017
1368-8375/© 2016 Elsevier Ltd. All rights reserved.

analogize a phase II clinical trial in terms of number of unique tumors studied [9]. While the largest cohorts of PDXs exist for breast, lung, colorectal, and pancreatic tumor types, a relatively small but growing number of HNSCC groups have been established. While characterization still differs from group to group, links between mutational profile and therapeutic response have now been investigated in several cases [4,10,11], and stability of proteomic markers from human to xenograft was evaluated in a single cohort [12].

Previously, our group established HNSCC PDXs from patients with both human papillomavirus (HPV) positive and HPV-negative cancers [1]. This initial work examined the stability in tumor histology and p16 expression across passages. Furthermore, we evaluated p53 and retinoblastoma expression of the PDXs to assess if this is related to HPV status and carried out initial chemoradiation experiments on a subset of PDXs. We subsequently determined that the time to re-implantation or storage solution used to house the tumor during the time delay did not have any impact on the maintenance of previously established PDXs [13].

In this work, we sought to further define our population of HNSCC PDXs, determine potential boundaries of this model and expand the future utility of this system. First, we continued to consent patients and expand our repertoire of PDXs and determine whether disease or demographic factors impacted PDX establishment rates. Next, we assessed whether the time (up to 24 h) from tumor excision in the operating room to implantation in the immunodeficient mice impacted initial PDX establishment, growth potential and histology. Finally, we evaluated the stability of both mutational and protein expression markers across PDX passaging. Using a targeted cancer mutation panel, we investigated the stability of mutations from the primary patient sample to multiple generations of PDX. Using quantitative IHC we determined whether any significant changes existed in the expression of putative predictive protein biomarkers across passaged PDXs. This work has important implications for the field of head and neck cancer research as it relates to the ongoing struggle to identify suitable predictive biomarkers to aid in the treatment of patients with HNSCC.

Materials and methods

Mice, PDX propagation, and tumor harvesting

Six to eight week old female NOD-SCID gamma (NSG, NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ) mice (Jackson Laboratories) were used for PDX establishment and amplification. UW-SCC1—36 PDXs were previously established and propogated in the lab [1]. New PDXs were generated and passaged in a similar manner; detailed methods are available in the Supplemental information.

New PDX establishment

Continued approval by the University of Wisconsin Institutional Review Board was obtained to discuss tissue donation with patients presenting to the clinic with newly diagnosed or recurrent HNSCCs. Consenting patients completed a form regarding tobacco/alcohol use, gender and age. At the time of surgery a small section of their tumor was obtained for PDX establishment in NSG mice as described previously [1]. Briefly, after receipt from the operating surgeon, tissue was mixed with a 1:1 mixture of media (Dulbecco's Modified Eagle Medium with 10% fetal bovine serum, 1% penicillin/streptomycin, and 2.5 μg/mL amphotericin B) and matrigel (catalog #354230, BD Biosciences, Inc) and minced into less than 1 mm^3 pieces. 100–200 μl of the mixture was injected subcutaneously into two flanks of two to four NSG mice through an 18-gauge needle such that all sites received roughly equivalent amounts of tumor tissue. We previously demonstrated that neither time nor storage solution impacted tumor growth potential nor histology in a subsequent passage for previously established PDXs [13]. We expanded on these initial findings by evaluating whether a 24 h time delay from initial excision in the operating room to ultimate implantation in the NSG mice impacted tumor take rate, growth potential, or histology for two new PDXs (UW-SCC63 and 64). After up to six months mice were sacrificed, any tumors harvested, weighed, and histological characteristics were evaluated by a board certified pathologist (C.Z.L). Additional details provided in supplemental methods.

Tissue microarray development and immunohistochemistry

A 196 core tissue microarray (TMA) was developed utilizing tissue from formalin fixed paraffin embedded (FFPE) tumors from each passaged PDX. All tumors were represented on the microarray by duplicate cores. The TMAs were sectioned (5 μm) and H&E stains were carried out on the thirtieth section. Remaining sections were stained by immunohistochemistry (IHC) for the expression of EGFR, pEGFR, pAkt, pERK and ERCC1 by standard IHC techniques [14], and detected with DAB. Additional details available in supplemental methods.

Tissue microarray analysis

The TMA was scanned by the UW TRIP lab's Vectra System and analyzed by inForm Software v1.4.0 (PerkinElmer, Waltham, MA). We followed a standardized approach to quantitatively evaluate the expression of the TMA cores for each biomarker [14]. The inForm Software outputs the DAB mean optical density (MOD) as the measure of expression of each marker as a continuous value from 0 to 1. To evaluate the stability of protein expression, the expression of each biomarker in the first and last passaged PDXs were compared. The DAB MOD values for all the PDXs were compared between the first and last passages using a paired *t*-test. All tests were two sided, and SAS/STAT software (version 9.4) was used to perform these analyses. Additional analyses were carried out for passaged PDXs with respect to pERK expression. For PDXs with more than two passages, all passages were compared via an one-way analysis of variance (ANOVA) test while for PDXs with only two passages a two sample *t*-test with equal standard deviations was used (Graphpad Prism v6.0d). For all statistical analyses a p-value less than 0.05 was considered statistically significant. Additional details in supplemental information.

Hotspot mutational analysis

To investigate the stability of mutations from the original patient tumor to the initial PDX and subsequent passages through mice we employed an amplicon based next generation sequencing cancer panel. Total genomic DNA was isolated from FFPE tissue and sequenced using the Illumina TruSeq Cancer Amplicon panel run on a MiSeq2000. The DNA sequencing reads were adapter and quality (Q20) trimmed and aligned to the reference genome, GRCh37. Variants were called using MuTect [15] version 1.4 followed by annotation with SnpEff [16]. Variants were further filtered by minimum allele frequency and annotated by comparing to published studies searchable on cBioPortal for Cancer Genomics http://www.cbioportal.org/ [17,18]. Additional method details in supplemental information.

Download English Version:

https://daneshyari.com/en/article/5642620

Download Persian Version:

https://daneshyari.com/article/5642620

Daneshyari.com