A DNA methylation-associated nomogram predicts the overall survival of osteosarcoma

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Abstract
Numerous reports have demonstrated that DNA methylation may be underlying prognostic biomarkers of cancer. However, few studies indicated that DNA methylation was independent biomarker for osteosarcoma prognosis. We aimed to discover and validate a novel DNA methylation signature for prediction of osteosarcoma patients’ overall survival (OS).

The DNA methylation data of osteosarcoma patients was researched from The Cancer Genome Atlas (TCGA) database. Overall, 80 samples with 485,577 DNA methylation sites were enrolled in our study. The 80 samples were randomly allocated into training dataset (first two-thirds) and validation dataset (remaining one-third). Initially, the univariate Cox proportional hazard analysis was performed in the training dataset to determine methylation sites significantly (P < 0.05) relevant to osteosarcoma patients’ OS as underlying indicators. Subsequently, the underlying indicators were employed to carry out the least absolute shrinkage and selection operator (LASSO) Cox regression analysis for further selecting the candidate methylation sites. Then, the selected candidate methylation sites were employed as covariates to perform multivariate Cox proportional hazard model for identifying the predictor of OS in osteosarcoma patients. The validation dataset was used to validate the predictive accuracy by receiver operating characteristic (ROC) analysis and Kaplan–Meier survival analysis.

We discovered a 7-DNA methylation signature closely relevant to OS of osteosarcoma patients. AUC at 1, 3, 5 years in training dataset (0.951, 0.922, 0.925, respectively), testing dataset (0.952, 0.918, 0.925, respectively), and entire dataset (0.952, 0.968, 0.968, respectively). Suggesting high predictive values for OS of osteosarcoma patients. In addition, a methylation-associated nomogram suggested good predictive value and clinical application.

We discovered and validated a novel 7-DNA methylation-associated nomogram for predicting OS of osteosarcoma patients.

Abbreviations: LASSO = least absolute shrinkage and selection operator, NA = not available, OS = overall survival, ROC = receiver operating characteristic curve, TGGA = The Cancer Genome Atlas.

Keywords: DNA methylation, nomogram, osteosarcoma, overall survival, The Cancer Genome Atlas.

1. Introduction
Osteosarcoma is the most common malignant bone tumor mainly developing in teenagers and young adults.

Osteosarcoma is highly aggressive and the 5-year survival rate of these osteosarcoma patients is 14%.

The survival rate has been greatly improved due to the application of neoadjuvant chemotherapy. Nevertheless, the prognosis of osteosarcoma patients with a poor response to chemotherapy is still dismal.

Assessment of the patients ahead of therapy might identify a risk-adapted method and may guide the development of improving personalized treatment, such as high-risk patients can be selected to novel therapies. The selection might promote the improvement of clinical trials which suggests clinical benefits. As we know, related molecular biomarkers could provide additional prognostic information and guide treatment selection for osteosarcoma.

Therefore, identifying effective prognostic signatures for overall survival (OS) of osteosarcoma patients is urgently required.

Numerous epigenetic studies suggested that gene methylation was a significant mechanism for occurrence and development of tumors.

The methylation usually results in the suppression of the promoter region, which hampers gene transcription and subsequently causes gene silencing.

Numerous reports have demonstrated that DNA methylation could serve as potential prognostic biomarkers. For example, it has been concluded that Iroquois homeobox 1 (IRX1) hypomethylation enhanced osteosarcoma metastasis and may be an underlying molecular marker.

OPCML gene promoter methylation may be an effective signature for predicting the prognosis for ovarian cancer....
patients. However, many recent osteosarcoma studies have several relatively small sample cohorts, lack of subsequent biomarker validation, concentration only on specimens with special clinical characteristics, or study of only a few genes. These studies lacked the combined and systematic research methods of genome-wide methylation analysis. Consequently, we analyzed the intact-genome methylation profiles of cancer tissues from osteosarcoma patients in The Cancer Genome Atlas (TCGA) database to determine DNA methylation markers for predicting osteosarcoma patients’ prognosis. The predictive ability of methylation signatures was evaluated with receiver operating characteristic (ROC) analysis and Kaplan–Meier survival analysis. In addition, a robust prognostic predicted ability was found in our nomogram for the prediction of osteosarcoma patients’ OS.

2. Materials and methods

2.1. DNA methylation data of osteosarcoma tissues

The osteosarcoma DNA methylation data measured with illumina Human Methylation 450 BeadChip (Illumina Inc., CA) and related clinical information was researched in TCGA database through R TCGAbiolinks package.[7] The coordinates of genome for the CpGs were implemented using GRCh38, β values were employed to stand for DNA methylation levels, computed as M/(M + U), U refers to the signal from unmethylated beads and, M refers to the signal from methylated beads at the targeted CpGsite. The data containing clinical survival information were selected for analyzing the relevance between DNA methylation levels and OS in osteosarcoma patients. Overall, 80 samples with 485,577 DNA methylation sites were enrolled in this study (Supplemental Digital Content [Table S1, http://links.lww.com/MD/F422]). These 80 samples were randomly divided into training dataset (first two-third) and validation dataset (remaining one-third). The training dataset was exploited for identifying and building prognostic hallmarks, and the validation dataset were applied for verifying the predictive robustness of the biomarker. This study was a secondary retrospective study. No ethical approval was required.

2.2. Data processing, normalization and identification of differentially expressed methylation sites

The data were preprocessed before developing the prediction model. Methylation sites whose beta value is not available (NA) in >10% of the total specimens were removed from our study. Then, the NA data was assumed through “impute.knn” function from Impute package.[8] Then, the data normalization was executed through “betaqin” function in wateRmelon package.[9]

Furthermore, all the specimens were assigned into metastasis cohort and non-metastasis cohort. The standardized beta was transformed to M value via the formulation: \( M = \log \left( \frac{b}{1-b} \right) \). M value was employed to eradicate the difference generated by different probes. Finally, M value was exploited to unearth differentially expressed methylation sites between metastasis and non-metastasis cohorts via “dmpFinder” function in minfi package.[10]

2.3. Statistical analyses

All of statistical analyses were executed based on the R statistical package (R version 3.6.1) except as otherwise noted. The follow up time of the osteosarcoma patients ranged from diagnosis to death. The univariate Cox proportional hazard analysis was first performed in the training dataset to identify methylation sites significantly (\( P < 0.05 \)) relevant to patients’ OS as underlying indicators. Subsequently, the underlying indicators were employed to carry out the least absolute shrinkage and selection operator (LASSO) Cox regression analysis for further selecting the candidate methylation sites. Then, the selected candidate methylation sites were employed as covariates to discover multivariate Cox proportional hazard model. Finally, a 7-DNA methylation signature was unearthed for predicting patients’ OS. A risk score formula was produced using the model to measure the prognostic risk score of each osteosarcoma patient. The patients were then divided into high- or low-risk cohorts across the median risk score. Then, the Kaplan–Meier estimator with log-rank test (Mantel–Cox) was implemented to measure the cumulative survival time and evaluates the differences in OS between the 2 groups. Kaplan–Meier curves were drawn based on the “survival” package.[11] Finally, the ROC analysis was performed to evaluate the model performance using the “pROC” package.[12]

2.4. Construction of the nomogram

Univariate and multivariate Cox model was implemented based on methylation associated risk score as well as several other clinicopathological factors to assess the independence of the 7-DNA methylation signature for predicting patients’ OS. Then, a nomogram that combined both the 7-DNA methylation signature-related risk score and the conventional clinicopathological factors was executed via the “rms” R package. C-index, ROC were calculated to evaluate the model performance of the nomogram.

### Table 1

| Characteristics         | Total | Training dataset (n=56) | Testing dataset (n=24) |
|-------------------------|-------|-------------------------|------------------------|
| Gender                  |       |training dataset (n=56)  | training dataset (n=24) |
| Female                  | 34(42.5) | 23 (41.08) | 11 (45.83) |
| Male                    | 46 (57.5) | 33 (58.92) | 13 (54.17) |
| Race                    |       |training dataset (n=56)  | training dataset (n=24) |
| White                   | 49 (61.25) | 38 (67.86) | 11 (45.83) |
| Asian                   | 7 (8.75) | 2 (3.57) | 5 (20.83) |
| Black or African American | 8 (10) | 5 (8.93) | 3 (12.5) |
| Unknown                 | 16 (20) | 11 (19.64) | 5 (20.83) |
| Ethnicity               |       |training dataset (n=56)  | training dataset (n=24) |
| Hispanic or Latino      | 9 (11.25) | 8 (14.29) | 1 (4.17) |
| Not Hispanic or Latino  | 52 (65) | 36 (64.29) | 16 (66.67) |
| Unknown                 | 19 (23.75) | 12 (21.43) | 7 (29.17) |
| Age                     |       |training dataset (n=56)  | training dataset (n=24) |
| <16                     | 49 (61.25) | 35 (62.5) | 14 (58.33) |
| >16                     | 31 (38.75) | 21 (37.5) | 10 (41.67) |
| Metastasis status       |       |training dataset (n=56)  | training dataset (n=24) |
| Metastatic              | 19 (23.75) | 13 (23.21) | 6 (25) |
| Non-metastatic          | 61 (76.25) | 43 (76.79) | 18 (75) |

The clinicopathological features of the included osteosarcoma patients.
exploited to measure the prognostic robustness of the nomogram. The result of the nomogram was showed in the calibrate curve, and 45° line suggested the perfect prediction ability.

3. Results

3.1. Clinical characteristics of the study populations

The study was implemented on 80 osteosarcoma patients who were clinically and pathologically diagnosed with osteosarcoma. Of these patients, 46 (57.5%) were men and 34 (42.5%) were women. The median age was 14.4 years (range, 3.6–32.4), respectively, and the median OS were 1387 days. The 3-year OS rate of all osteosarcoma patients was 52.5%. Specific tumor site list included arm, femur, fibula, humerus, ilium, leg, radius, tibia. Femur group (36 samples) served as the most common type (45%). Race list included White, Asian, Black or African American, Unknown group. White group (49 samples) was the most common race (61.25%). The clinicopathological features of the included osteosarcoma patients were exhibited in Table 1. The flow chart of the present study was display in Fig. 1.

3.2. Identification of 7 methylation sites signature

The 2503 differentially expressed methylation sites were assessed between metastasis group and no metastasis group. A total of 237 DNA methylation sites were revealed to be strongly correlated with the OS of osteosarcoma patients via univariate Cox proportional hazard regression model ($P < .01$). Subsequently, LASSO Cox regression model were implemented using the 237 DNA methylation sites and 12 methylation sites were selected as the candidate prognostic factors for predicting OS of osteosarcoma patients (Fig. 2A and B). Subsequently, multivariate Cox proportional hazard regression analysis were carried out using the 12 DNA methylation sites, and a combination of the 7 methylation sites (cg04160915, cg22597058, cg17630044, cg09736950, cg02176678, cg16570917, cg10468845) was selected as the optimum model for predicting OS of osteosarcoma patients. The risk score formula of the 7 methylation sites was discovered: Risk score = $-0.404 \times cg16570917 - 0.426 \times cg22597058 + 0.373 \times cg02176678 - 0.711 \times cg10468845 - 0.879 \times cg17630044 + 2.171 \times cg04160915 - 1.253 \times cg09736950$. Obviously, the hypermethylation levels of cg02176678

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**Figure 1.** Flow chart of the bioinformatics analysis process.
and cg04160905 were in accordance with a higher risk, meanwhile, the hypomethylation levels of cg16570917, cg22597058, cg10468845, cg17630044 and cg09736950 were in accordance with a higher risk (Fig. 3). The genes corresponding with these 7 sites were DENND1B, EP400, MGC15885, TLN2, TTLL4, PTPRF, C3orf31.

3.3. Association between 7-DNA methylation signature and osteosarcoma patients’ OS in the training, validation, and the entire datasets

The patients were then divided into high- or low-risk cohorts with the median risk score. The Kaplan–Meier analysis was exploited in the testing and training datasets as well as entire dataset to examine the difference of osteosarcoma patients’ OS in the low- versus high-risk group. The OS of high-risk patients tended to be shorter than that of low-risk patients (P=.048) (Fig. 4A) in training group, a similar result was exhibited in the testing dataset (P=4e-7) (Fig. 4C) and entire dataset (P=4e-7) (Fig. 4E). These results suggested that the 7-DNA methylation signature could stratify patients into high- and low-risk cohorts, implying its potential clinical utility in predicting osteosarcoma prognosis.

3.4. Evaluation of the predictive performance of the 7-DNA methylation signature by using ROC analysis

The AUC values of the ROC curves were employed for evaluating the power of the 7-DNA methylation signature in predicting osteosarcoma patients’ OS. The AUC of the 7-DNA methylation signature at 1, 3, 5 years in training dataset (0.952, 0.968, 0.968),
respectively (Fig. 4B). A good predictive robustness was also found in testing dataset (0.952, 0.918, 0.925), respectively, (Fig. 4D) and entire dataset were 0.951, 0.922, 0.925, respectively, (Fig. 4F), implying that the 7-DNA methylation signature had good power, and has great potential to function as a prognostic hallmark in clinical applications.

In addition, patients were ranked through their risk scores (Fig. 5A), and the dotplot was implemented via their survival status (Fig. 5B), supporting that the patients in the high risk group had a poorer prognosis than those in the low risk group. Heatmap of 7 methylation sites grouped by risk score was showed in Fig. 5C, which was in accordance with our above result. Following that, we implemented subgroup analysis using some clinic-related variables including age, sex, treat, race, and metastasis status. Most of subgroups showed that the 7-DNA methylation signature was an accurate classifier for osteosarcoma patients’ OS (Supplemental Digital Content [Fig. S1–5, http://links.lww.com/MD/F423]).
3.5. Nomogram development

To assess the independence of the 7-DNA methylation signature for predicting osteosarcoma patients’ OS, univariate, and multivariate Cox model was implemented based on methylation associated risk score as well as several other clinicopathological factors. Hazard ratios (HRs) suggested that the 7-DNA methylation signature was closely linked to the OS of osteosarcoma patients ($P < .001$, HR 2.86, 95% CI 2.05–3.99) (Table 2), demonstrating that the signature served as an independent prognostic hallmark. To improve the prognostic model's predicted ability in a quantitative method, we developed a nomogram (Fig. 6) that combined both the 7-DNA methylation signature and the conventional clinicopathological factors. The importance of the factors was displayed in Fig. 7A. The result showed that the 7-DNA methylation signature-associated nomogram had a high value for predicting OS of patients with osteosarcoma. The evaluative elements including C-index (0.911,

Table 2

| ID   | Univariate Cox analysis | Multivariate Cox analysis |
|------|-------------------------|---------------------------|
|      | HR | HR.95L | HR.95H | $P$ value | HR | HR.95L | HR.95H | $P$ value |
| Score | 2.718282 | 2.055113 | 3.59545 | 2.41E–12 | 2.863886 | 2.05197 | 3.997057 | 6.18E–10 |
| Gender | 0.966358 | 0.44357 | 2.1053 | 0.931359 | 0.721949 | 0.254356 | 2.04914 | 0.540471 |
| Race | 1.057636 | 0.70996 | 1.575572 | 0.782895 | 0.98452 | 0.660451 | 1.467604 | 0.938951 |
| Ethnicity | 1.355486 | 0.784064 | 2.343358 | 0.276156 | 1.275542 | 0.611505 | 2.660659 | 0.516468 |
| Age | 0.999927 | 0.99968 | 1.000174 | 0.563658 | 1.00002 | 0.999694 | 1.000347 | 0.903204 |
| Metastasis | 4.261302 | 1.958595 | 9.271286 | 0.000257 | 0.803814 | 0.276137 | 2.339846 | 0.688714 |
| Site | 0.6194 | 0.367198 | 1.04482 | 0.072557 | 0.66886 | 0.363786 | 1.229771 | 0.195551 |

Methylation associated risk score and several other clinicopathological elements.
95% CI: 0.866–0.956) and AUC (0.951, 0.922, 0.925) (Fig. 7B), which demonstrated a promising clinical prospect.

4. Discussion

It has reported that molecular signatures can predict the clinical prognosis in various tumors.[13–16] For example, GBX2 methylation serves as a novel prognostic biomarker and improves prediction ability of biochemical recurrence among patients with prostate cancer negative for intraductal carcinoma and cribriform architecture.[17] DNA methylation of CRB3 functions a prognostic signature for clear cell renal cell cancer.[18] Whereas, many of these investigations were limited by either less sample or lacking availability of the hallmark as an independent prognostic signature. Some studies suggested that combinations of DNA methylation as signatures may achieve good power than individual DNA methylation.[19] In this study, a 7-DNA methylation signature closely relevant to the OS of osteosarcoma patients was identified according to genome-wide DNA methylation comprehensive analysis. The ROC analysis suggested that the 7-DNA methylation signature had a robust power in predicting osteosarcoma patients’ OS. The 7-DNA methylation signature also acted well in distinguishing low- and high-risk cohorts based on the Kaplan–Meier analysis with crucial P values, indicating that it was a robust predictor of osteosarcoma patients’ OS.

The selected 7 methylation sites were projected into 7 genes: DENND1B, EP400, MGC15885, TLN2, TTLL4, PTPRF, C3orf31. Researchers have reported that the above 7 genes may be important in cancer progression. For example, Cotterchio et al.[20] reported that DENND1B was significantly related with pancreas cancer risk. Kashiwaya et al.[21] suggested that TTLL4 could play significant roles in pancreatic carcinogenesis via its polyglutamylase activity and following coordination of chromatin remodeling, and might be a novel molecular candidate for the application of new therapeutic methods for pancreatic cancer.
PTPRF expression has been identified as a potential prognostic/predictive marker for treatment with erlotinib in non-small-cell lung cancer.\textsuperscript{[22]} A research has reported that genome-wide siRNA screen identifies SMCX, EP400, and Brd4 as E2-dependent regulators of human papillomavirus oncogene expression.\textsuperscript{[23]} Both talin-1 and talin-2 were correlated with malignancy ability of the human hepatocellular cancer MHCC-97 L cell\textsuperscript{[24]} which suggested the key role of TLN2 in cancer development. In spite of the functional mechanism of these 7 genes remains to be fully explored, their methylation has important connections with the prognosis of patients with osteosarcoma and may function as an underlying therapeutic target for osteosarcoma.

Limitations exist in our study. Firstly, no external validation set was employed to verify the predictive value of the 7-DNA methylation signature for osteosarcoma patients’ OS, which may yield some sort of biases. Secondly, in our study, the number of osteosarcoma patient is limited and our research is retrospective one, thus, more prospective researches containing more samples from various medical centers were required to test the predictive power of this signature. Thirdly, genome-wide methylation measurements for the above prospective researches are needed before this model is used in the clinic. In spite of the above limitations, there are still a few significant points. In the present study, we exploited LASSO method to eradicate difference between univariate and multivariate Cox analysis, which perfectly eradicated the multicollinearity effect and made our conclusion more reliable. Besides, few previous researches have integrated methylation hallmark with clinical factors to predict OS of osteosarcoma patients. And no study was employed as above for osteosarcoma so far. Furthermore, a nomogram was developed based on the 7-DNA methylation signature and several other clinicopathological factors, offering novel method for clinical prediction. Meanwhile, C-index and ROC performed well in our model, which suggested that our nomogram can successfully improve predicted ability in OS of osteosarcoma patients.

5. Conclusion

In conclusion, according to genome-wide comprehensive analysis of DNA methylation data for 80 osteosarcoma patients, this study revealed that a 7-DNA methylation signature was importantly associated with OS of patients with osteosarcoma, and the predictive value of the 7-DNA methylation signature for osteosarcoma patients’ OS was verified by ROC analysis and Kaplan–Meier survival analysis. The result concluded that the 7-DNA methylation signature may be independent prognostic hallmark and may be a key tool for guiding the clinical therapy of osteosarcoma patients. In addition, the result suggested that our nomogram can successfully improve predicted ability in OS of osteosarcoma patients.
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References

[1] Lindsey BA, Markel JE, Kleinerman ES. Osteosarcoma overview. Rheumatol Ther 2017;4:25–43.
[2] Hutanu D, Popescu R, Stefanescu H, et al. The molecular genetic expression as a novel biomarker in the evaluation and monitoring of patients with osteosarcoma-subtype bone cancer disease. Biochem Genet 2017;55:291–9.
[3] Yuan H, Gao Y. MicroRNA-1908 is upregulated in human osteosarcoma tissues and regulates cell proliferation and migration by repressing PTEN expression. Oncol Rep 2015;34:2706–14.
[4] Lo KW, Huang DP. Genetic and epigenetic changes in nasopharyngeal carcinoma. Semin Cancer Biol 2002;12:451–62.
[5] Geiman TM, Robertson KD. Chromatin remodeling, histone modifications, and DNA methylation-how does it all fit together? J Cell Biochem 2002;87:117–25.
[6] Lu J, Song G, Tang Q, et al. IRX1 hypomethylation promotes osteosarcoma metastasis via induction of CXCL14/NF-kappaB signaling. J Cell Investig 2015;12(5):1839–56.
[7] Zhou F, Tao G, Chen X, et al. Methylation of OPCML promoter in ovarian cancer tissues predicts poor patient survival. Clin Chem Lab Med 2014;52:735–42.
[8] Janssen KJ, Donders AR, Harrell FE Jr, et al. Missing covariate data in medical research: to impute is better than to ignore. J Clin Epidemiol 2010;63:721–7.
[9] Pidday R, CC YW, Volta M, et al. A data-driven approach to preprocessing Illumina 450K methylation array data. BMC Genomics 2013;14:293.
[10] Aryee MJ, Jaffe AE, Corrada-Bravo H, et al. Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. Bioinformatics 2014;30:1363–9.
[11] Durisova M, Dedik L. SURVIVAL—an integrated software package for survival curve estimation and statistical comparison of survival rates of two groups of patients or experimental animals. Methods Find Exp Clin Pharmacol 1993;15:535–40.
[12] Rabin X, Turck N, Hainard A, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics 2011;12:77.
[13] Groes L, Beyens M, Fransen E, et al. Large-scale analysis of DNA methylation reveals its potential as biomarker for breast cancer. Clin Epigenetics 2018;10:51.
[14] Chou JL, Huang RL, Shay J, et al. Hypermethylation of the TGF-beta target, ABCA1 is associated with poor prognosis in ovarian cancer patients. Clin Epigenetics 2015;7:1.
[15] Jin C, Xue Y, Li Y, et al. A 2-protein signature predicting clinical outcome in high-grade serous ovarian cancer. Int J Gynecol Cancer 2018;28:391–8.
[16] Zhang T, Guan G, Chen T, et al. Methylation of PCDH19 predicts poor prognosis of hepatocellular carcinoma. Asia Pac J Clin Oncol 2018;14:935–7.
[17] Jeyapala R, Savio AJ, Olinkov-Mitsel E, et al. GBX2 methylation is a novel prognostic biomarker and improves prediction of biochemical recurrence among patients with prostate cancer negative for intra-ductal carcinoma and cribriform architecture. Eur Urol Oncol 2019;2:231–8.
[18] Li P, Liu J, Li J, et al. DNA methylation of CRB3 is a prognostic biomarker in clear cell renal cell carcinoma. Mol Biol Rep 2019;46:4377–83.
[19] Dai W, Teodoridis JM, Zeller C, et al. Systematic CpG islands methylation profiling of genes in the wnt pathway in epithelial ovarian cancer identifies markers of progression-free survival. Clin Cancer Res 2011;17:4052–62.
[20] Gotterchio M, Lowcock E, Bidner-Canfield Z, et al. Association between variants in atopy-related immunologic candidate genes and pancreatic cancer risk. PLoS One 2015;10:e0125273.
[21] Kashywaya K, Nakagawa Y, Hossokawa M, et al. Involvement of the tubulin tyrosine ligase-like family member 4 polyglutamylation and chromatin remodeling in pancreatic cancer cells. Cancer Res 2010;70:4372–8.
[22] Soulieres D, Hirsch FR, Shepherd FA, et al. PTPRF expression as a potential prognostic/predictive marker for treatment with erlotinib in non-small-cell lung cancer. J Thorac Oncol 2013;10:1364–9.
[23] Smith JA, White EA, Sowa ME, et al. Genome-wide siRNA screen identifies SMCX, EP400, and Brd4 as E2-dependent regulators of human papillomavirus oncogene expression. Proc Natl Acad Sci U S A 2010;107:3752–7.
[24] Fang KP, Dai W, Ren YH, et al. Both Talin-1 and Talin-2 correlate with malignancy potential of the human hepatocellular carcinoma MHCC-97 L cell. BMC Cancer 2016;16:45.