—Review—

Development of Biomarkers to Predict Recurrence by Determining the Metastatic Ability of Cancer Cells

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Adjuvant chemotherapy is administered to cancer patients after curative resection but is unnecessary when patients without micro-metastatic lesions undergo a perfectly curative surgical procedure. Patients who need adjuvant chemotherapy are those with micro-metastases outside the resection area that are not detectable by imaging, despite curative resection at primary sites. If biomarkers that reflect metastatic potential could be developed, personalized adjuvant chemotherapy could be provided in clinical settings. Actinin-4 (ACTN4, gene name *ACTN4*) is an actin-bundling protein identified in 1998 as a novel molecule involved in cancer invasion and metastasis. Overexpression of actinin-4 protein in cancer cells leads to an invasive phenotype, and patients with gene amplification of *ACTN4* have a worse prognosis than patients with a normal copy number for cancers of the pancreas, lung, and salivary glands, among others. This review summarizes the biological roles of actinin-4 in cancer invasion and metastasis and examines the potential usefulness of actinin-4 as a biomarker for evaluation of metastatic ability. (J Nippon Med Sch 2022; 89: 24–32)

Key words: biomarker, metastatic ability, actin-bundling protein, *ACTN4*

Introduction

Control of metastatic sites is important in determining the prognosis of patients after curative resection at primary sites. Despite curative surgery, some cancers recur at sites other than those of the primary lesions[1,2]. Even if the primary tumor is surgically resectable at the macroscopic level, if cancer cells have a high inherent metastatic ability, the presence of micro-metastases that cannot be detected by imaging modalities cannot be ruled out, and it may be difficult to determine the optimal therapeutic strategy after complete resection[3]. In general terms, patients who might derive a potential benefit from adjuvant chemotherapy are defined as those having the potential for micro-metastatic lesions in the other sites of the resected area and those who are highly sensitive to the drug used in adjuvant chemotherapy. Under the minimum assumption that patients for whom adjuvant chemotherapy is potentially beneficial are defined as persons at high risk of metastasis, I developed a biomarker to evaluate metastatic potential. Gene amplification of *ACTN4* is a potential biomarker for optimizing the therapeutic strategy for adjuvant chemotherapy.

Identification of Actinin-4, Which is Associated with Cancer Invasion and Metastasis

To identify the molecular mechanism of cancer invasion, I established a new monoclonal antibody (NCC-Lu-632), which exhibits a strong reaction to the invasive front of cancer cells, and then performed molecular cloning for the antigen of NCC-Lu-632 by using a phage screening assay. I isolated the novel isoforms of alpha-actinin, that is the actin-bundling protein, and named it actinin-4 (ACTN4)[4].

Alpha-actinin consists of 4 isoforms (ACTN1-4) in humans[5]. ACTN1 and ACTN4 are classified as non-muscle type, and ACTN2 and ACTN3 are muscle type. The amino acid sequences of ACTN isoforms maintain high homology. Alpha-actinin has an actin-binding domain (ABD) comprising 2 calponin homology domains, 4 spectrin repeats (SRs), and 2 EF-hand domains, which include

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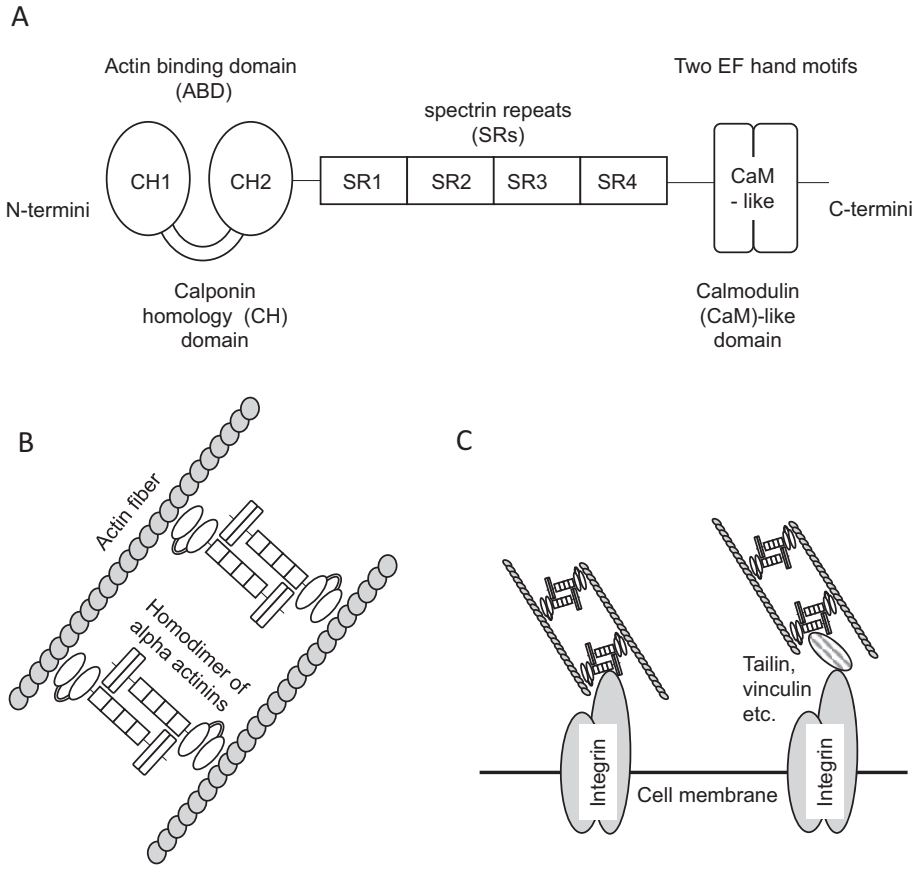

Fig. 1 Schema of actinin-4 as an actin-bundling protein[3].
(A) Domain structure of actinin-4 protein. Actinin-4 consists of the actin binding domain (ABD), spectrin repeat (SR1-4), and calmodulin (CaM)-like domain (two EF hand motifs).
(B) Actin bundling with actinin-4 homodimers. Alpha-actinin forms anti-parallel homodimers via SRs, which allows both sides of the ABD to bind actin filaments.
(C) Interaction of actinin-4 with the cell membrane. Actinin-4 interacts with actin filaments to bind the plasma membrane through beta 1-3 integrins, vinculin, and alpha-catenin.

calmodulin (CaM)-like domains. Alpha-actinin forms anti-parallel homodimers via the SRs; thus, both sides of the ABD can bind actin filaments and then do actin-bundling. Moreover, non-muscle alpha-actinins interact with actin filaments to bind with the plasma membrane through beta 1-3 integrins, vinculin, and alpha-catenin (**Fig. 1**)[3].

Immunohistochemical studies have shown that actinin-4 protein is histologically concentrated at the invasive fronts of several cancers, including colorectal[6,7], lung[8–11], breast[4,12], and ovarian cancers[13–15]. I previously reported that patients with protein overexpression of actinin-4 had worse outcomes than those without such overexpression who had cancers of the breast[4,12], pancreas[16,17], ovaries[13–15], thyroid[18], salivary gland[19], and tongue[20]. Moreover, involvement of actinin-4 protein overexpression was reported in malignant phenotypes of brain tumors[21–23], head and neck cancer[24,25], lung cancer[26–28], breast cancer[29–31], esophageal cancer[32], gastric cancer[33], pancreatic cancer[34], gastrointestinal stromal tumor (GIST)[35], cervical cancer[36], ovarian cancer, bladder cancer[37], prostate cancer[38,39], melanoma[40,41], leukemia[42,43], and osteosarcoma[44,45]. In fact, use of an exogenous transfection technique to overexpress actinin-4 in cancer cells revealed that cancer cells overexpressing actinin-4 can form protrusions that are involved in cell motility and cancer invasion and exhibit significantly increased invasive potential[6]. Moreover, reduction of actinin-4 protein expression decreased cell invasiveness in studies using RNA interference in pancreatic cancer[16], oral cancer[24], and lung cancer[10]. Thus, I concluded

that overexpression of actinin-4 is involved in cancer invasion and metastases.

Identification of Gene Amplification of *ACTN4* in Invasive Phenotypes of Malignant Tumors

In 2000, Kaplan et al. suggested that mutations in the gene encoding actinin-4 (*ACTN4*) were the cause of disease in 3 families with autosomal-dominant forms of familial focal segmental glomerulosclerosis (FSGS), after genotyping family members at markers on chromosome 19q13[46,47]. The locus of human chromosome 19q13 is often amplified in patients with cancers such as pancreatic cancer and ovarian cancer. I confirmed the amplification status of *ACTN4* in resected pathological specimens, using a fluorescence in situ hybridization (FISH) probe that was newly isolated in those studies. First, gene amplification of *ACTN4* was identified in pancreatic cancer[16]. Overexpression of actinin-4 protein was confirmed in almost all patients by gene amplification of *ACTN4* by immunohistochemistry (IHC), and the results of IHC and FISH analyses were significantly correlated in patients undergoing gene amplification of *ACTN4*. However, the cases of actinin-4 protein overexpression were not always gene amplification cases of *ACTN4*. Interestingly, Yamamoto et al. reported that gene amplification of *ACTN4* is a significant predictor of overall survival in patients with ovarian cancer and that an increase in *ACTN4* copy number was a more accurate predictor than IHC evaluation of ovarian cancer outcome[14]. Similar results have been observed for lung adenocarcinoma[8], tongue cancer[20], and salivary gland cancer[19].

Development of a Biomarker That Efficiently Stratifies Patients with Lung Adenocarcinoma Who Need Adjuvant Chemotherapy

The Japanese Lung Cancer Society Guideline for non-small cell lung cancer (2020) strongly recommends complete resection of postoperative pathological stage IA/IB/II (Group 8) lesions with an overall diameter of >2 cm and lung adjuvant chemotherapy with a tegafur-uracil combination (UFT) for patients with stage I adenocarcinoma of the lung (Evidence level A). The rationale for this recommendation is that a phase III trial of the efficacy of UFT for stage I lung adenocarcinoma showed an additive effect of 3 percentage points (85% to 88%) overall and 11 percentage points (74% to 85%) in stage IB (T > 3 cm)[48]. A meta-analysis of 4 additional clinical trials (2003 cases; 84% adenocarcinoma, 16% non-adenocarcinoma) showed an overall improvement in 5-year survival of 5 percentage points (77% to 82%), confirming the efficacy of UFT[49]. A subgroup analysis of patients with a tumor size of >2 cm and ≤3 cm showed an additional improvement in 5-year survival of 6 percentage points in that subgroup, with a hazard ratio (HR) of 0.62 [95% confidence interval (95% CI) 0.42-0.90], which was described as favorable[50]. The guideline further states that 74% of patients with complete resection of postoperative stage I disease (lung adenocarcinoma) are recurrence-free after surgery alone and that chemotherapy safety should be carefully considered when clinical oncologists administer adjuvant chemotherapy (The Japanese Lung Cancer Society Guideline 2020).

As mentioned above, assessment of tumor metastatic activity to predict micro-metastases outside the resection site is likely to be an important indicator for stratifying patients who respond to adjuvant chemotherapy and for selecting effective adjuvant chemotherapy.

Therefore, using immunostaining and FISH, I confirmed actinin-4 protein expression and gene amplification in resected specimens of stage I lung adenocarcinoma that had been collected at the National Cancer Center Hospital and National Cancer Center East Hospital from patients who did not receive postoperative adjuvant chemotherapy[11]. Using 2 independent cohorts, I analyzed the clinical utility of ACTN4 as a biomarker. Overall survival was significantly shorter for patients with stage I lung adenocarcinoma with gene amplification of ACTN4 (amplification group) (n = 23) than for the normal copy number group (267 patients): the 5-year survival rate was 95% (95% CI 82% to 98%) in the gene amplified group and 57% in the amplification-negative group (**Fig. 2A**). On univariate analysis, the HR for death was significantly higher in the amplification group than in the normal copy number group (HR 10.5; 95% CI 4.15-26.7), and multivariate analysis showed an HR of 6.78 (95% CI 2.59-17.7), which was extracted as a significant, independent, and the strongest, prognostic factor. Furthermore, I classified the patients in another independent cohort into 3 groups for analysis of overall survival, as follows: i) ACTN4 protein-negative group/normal copy number group; ii) ACTN4 protein-positive group/normal copy number group; and iii) ACTN4 protein-positive group/amplification group. The 5-year survival rates for the ACTN4 protein-negative group/normal copy number group (98 cases), ACTN4 protein-positive group/normal copy number group (88 cases), and ACTN4 protein-positive group/gene amplification group (19 cases) were 96% (95% CI 92% to 100%), 93% (95% CI 81% to 95%),

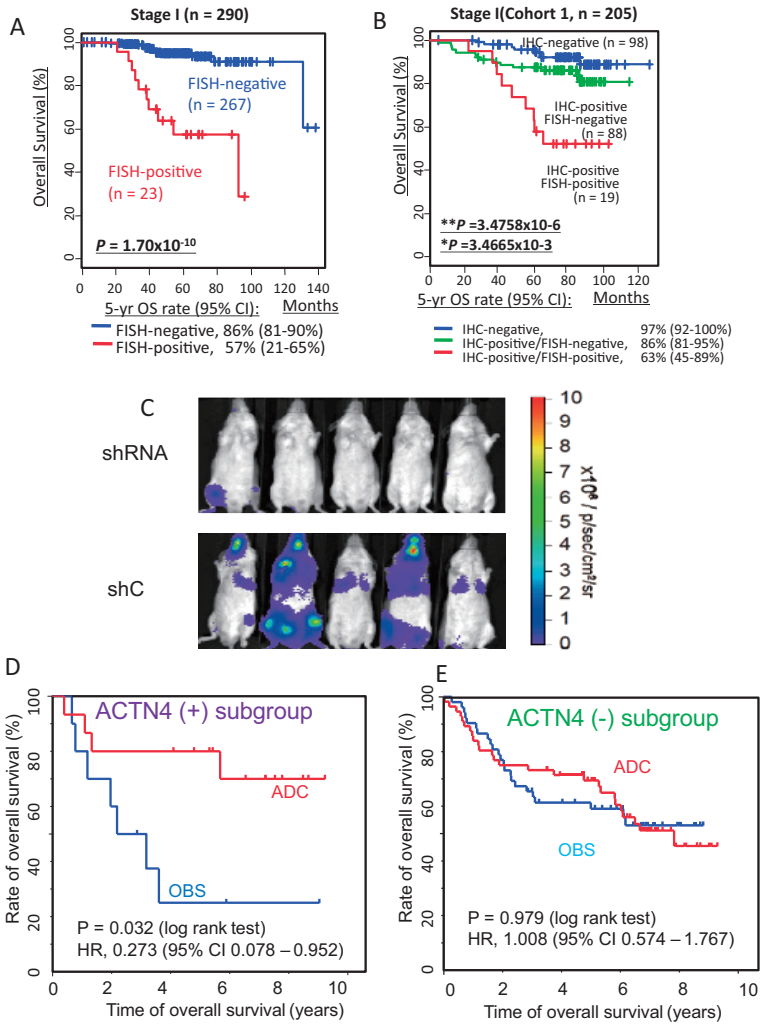

Fig. 2 Involvement of actinin-4 in metastatic potential of non–small cell lung cancer[8, 10].
(A) Prognostic significance of ACTN4 gene amplification in adenocarcinoma of the lung, as determined by Kaplan-Meier analysis of overall survival of patients with stage I adenocarcinoma of the lung. The blue line indicates the patients with normal copy numbers (FISH negative). The red line indicates patients with gene amplification of *ATCN4*[8].
(B) Using combination IHC and FISH to predict survival for patients with stage I adenocarcinoma of the lung. Overall survival was estimated by Kaplan-Meier analysis. The blue line indicates absence of actinin-4 protein expression (IHC-negative). The green line indicates strong expression of actinin-4 protein (IHC-positive) and a normal copy number of *ACTN4* (IHC-positive/FISH-negative). The red line indicates strong expression of actinin-4 protein (IHC-positive) and gene amplification of *ACTN4* (IHC-positive/FISH-positive)[8].
(C) Effect of ACTN4 knockdown by shRNA on the metastatic ability of a lung cancer cell line with gene amplification of *ACTN4* in an animal inoculation model[10].
(D-E) Overall survival curves from a reanalysis of publicly available data on patients enrolled in JBR.10. Subgroup with ACTN4 overexpression (D), and subgroup without ACTN4 overexpression (E). The red line indicates overall survival for patients who underwent adjuvant chemotherapy (ADJ). The blue line indicates the patients who were observed but did not undergo adjuvant chemotherapy (OBS)[10].

and 63% (95% CI 45% to 89%), respectively (**Fig. 2B**)[8]. These findings strongly suggest that not only is *ACTN4* gene amplification a strong prognostic biomarker for stage I lung adenocarcinoma in patients who did not undergo postoperative chemotherapy, it is strongly associated with mortality, despite complete resection. Thus, *ACTN4* gene amplification appears to be a biomarker for predicting minimal residual metastases that cannot be detected by imaging modalities[3].

In fact, when I knocked down expression of actinin-4 protein by using shRNA in A549 cells, a lung cancer cell line that showed gene amplification of *ACTN4*, the invasive ability and filopodia formation of cells were markedly reduced. When the luciferase luminescent A549 cell line was injected into the tail vein of immunodeficient mice and observed for 40 days, numerous metastatic lesions were observed in the lung. In contrast, no metastatic lesions were observed in the lungs of mice injected with the actinin-4 knockdown A549 cell line (**Fig. 2C**)[10]. In other words, as hypothesized previously, *ACTN4* gene amplification is likely to be a surrogate biomarker that reflects the presence of micro-metastases outside the resected area, which result from increased metastatic activity.

The Japanese Lung Cancer Society Guideline for non-small cell lung cancer (2020) CQ29 recommends cisplatin combination chemotherapy for complete resection of stage II-IIIA postoperative pathological disease (Group 8). The rationale for this recommendation is the findings of a meta-analysis showing that adjuvant chemotherapy significantly prolongs survival[51]. Using a publicly available database of results from the Canadian phase III, randomized, controlled trial of adjuvant cisplatin-vinorelbine (JBR.10)[52], a subgroup analysis of the ACTN4 mRNA index showed that postoperative adjuvant chemotherapy prolonged overall survival in the ACTN4 high-expression group (**Fig. 2D**) but not in the ACTN4 low-expression group. In the ACTN4 high-expression group, postoperative adjuvant chemotherapy prolonged overall survival, whereas in the ACTN4 low-expression group, no improvement in survival was observed (**Fig. 2E**). These results suggest that ACTN4 is a potential biomarker for stratifying patients who would benefit from adjuvant chemotherapy. On the basis of these results, an investigator-initiated study of the implementation of this biomarker was initiated by a multicenter collaboration of Nippon Medical School, Tokyo Medical University, and the National Cancer Center, with support from the Japan Agency for Medical Research and Development (AMED) (principal investigator Professor Kubota, Nippon Medical School).

Gene Amplification of *ACTN4* Strongly Predicts Outcomes of Tongue Squamous Cell Carcinoma

The current standard surgical treatment for stage I/II tongue cancer without clinical lymph node metastasis is partial tongue resection or partial tongue resection plus neck dissection on the affected side. The clinical question is whether elective neck dissection is necessary for early-stage tongue cancer. A 2015 randomized phase III trial from India provided one answer to this question: elective neck dissection for early-stage oral cancer was superior to non-dissecting surgery in the primary analysis of overall survival (HR 0.64 95% CI 0.34-0.59; 3-year survival rate 80.0% vs. 67.5%) and in the secondary analysis of disease-free survival (HR 0.45; 95% CI 0.34-0.59; 3-year disease-free survival rate 69.5% vs. 67.5%)[53]. It is questionable whether these results can be extrapolated to daily practice in Japan.

To resolve this question, a randomized, comparative study was conducted in Japan to evaluate the value of omitting prophylactic neck dissection for stage I/II tongue cancer (JCOG 1601: Randomized Phase III study to evaluate the value of omission of prophylactic neck dissection for stage I/II tongue cancer)[17,54]. This study was designed to evaluate the noninferiority in overall survival of partial tongue resection plus prophylactic dissection group, as compared with partial tongue resection, and to establish a less invasive standard of care. The study is ongoing.

Late cervical lymph node metastasis is likely caused by the metastatic activity of the primary tumor. If this is true, amplification of the *ACTN4* gene may be a potential biomarker for later cervical metastasis. In a recent study, I retrospectively examined protein expression and gene amplification of *ACTN4* by immunostaining and FISH, using stage I/II surgical pathological sections from the Department of Head and Neck Surgery, National Cancer Center Hospital. Patients were classified as i) negative for immunostaining (negative for actinin-4 protein); ii) positive for immunostaining (positive for actinin-4 protein) (**Fig. 3A**)/negative for FISH (normal copy number of ACTN4 gene); and iii) positive for immunostaining/FISH (amplified *ACTN4* gene) (**Fig. 3B**). Disease-free survival and overall survival were then evaluated. The study included 54 patients who underwent partial resection of the tongue at the first surgery at the National Cancer Center Hospital. Patients were excluded if they had un-

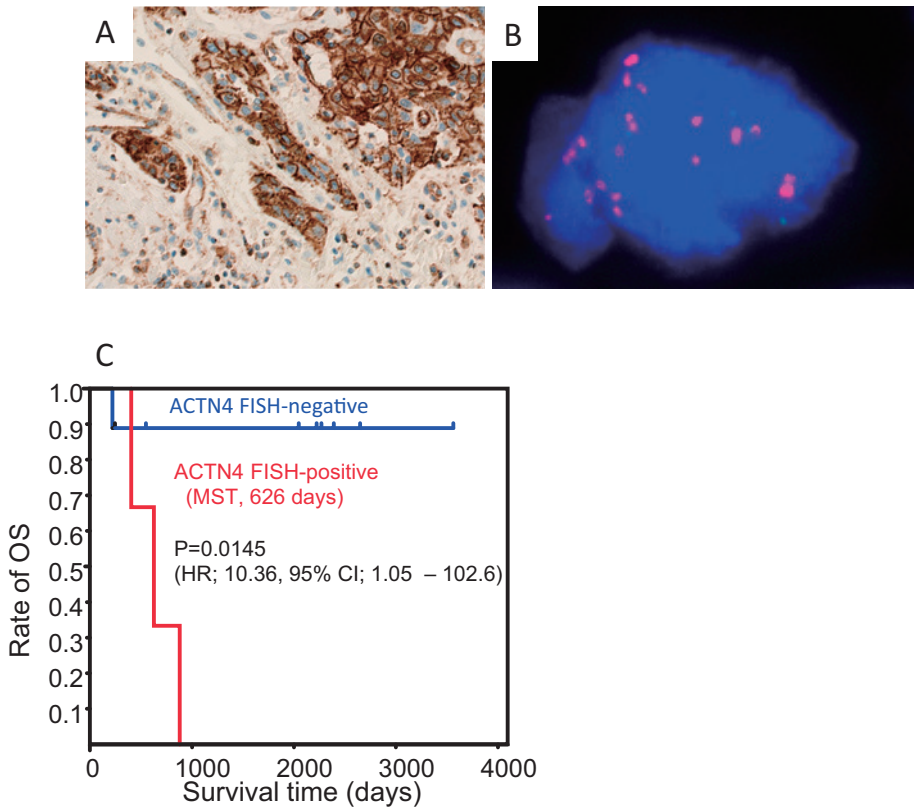

Fig. 3 Potential biomarker for stratification of patients with late metastatic ability of cervical lymph nodes in tongue cancer[20].
(A, B) Representative photograph of strong expression of actinin-4 protein (A) and gene amplification of *ACTN4* (B) in patients with stage I/II tongue cancer[20].
(C) Overall survival curves for 12 patients who underwent therapeutic neck dissection for late cervical lymph node metastases after undergoing partial glossectomy. The blue line is patients with a normal copy number of *ACTN4*. The red line is gene amplification of *ACTN4*[20].

dergone preoperative chemotherapy, had pathologically positive resection margins, had developed local recurrence, or could not be evaluated by FISH. Disease-free survival was significantly longer in patients who were i) immunostaining negative than in those who were ii) immunostaining positive/FISH negative (HR 2.82, 95% CI 1.04-7.64) and iii) immunostaining positive/FISH positive (HR 2.19, 95% CI 1.17-4.09). The median survival time (MST) was 1,964 days and 744 days for ii) immunostaining positive/FISH negative and iii) immunostaining positive/FISH positive, respectively; MST was not reached for the i) immunostaining negative group. Interestingly, when overall survival was evaluated in the same patients, the significant difference between i) immunostaining negative and ii) immunostaining positive/FISH negative disappeared, and the difference between i) immunostaining negative and iii) immunostaining positive/FISH positive (HR 7.60, 95% CI 1.95-29.6, p=0.0008), ii) immunostaining positive/FISH negative, and iii) immunostaining positive/FISH positive (HR 4.62, CI 0.998-21.3, P=0.035) were recognized[18]. These results suggest that actinin-4 protein-positive cases with normal *ACTN4* copy numbers can be rescued by second-line therapy, even after recurrence. Therefore, I investigated overall survival in 12 patients who underwent cervical dissection after recurrence at the National Cancer Center Hospital and found that the MST of ACTN4-amplified patients was 626 days, which was extremely short, and that all but one of the patients with a normal copy number could be rescued[20] (**Fig. 3C**). Because this was a retrospective

study with a small number of cases, it requires validation by multicenter studies, including retrospective and prospective observational studies.

Summary

Research that began with gene cloning has shown potential as a method to determine biomarkers for identification of groups at high risk for cancer recurrence and as indicators for implementation of adjuvant therapy. However, many hurdles must be overcome before implementation in clinical practice. I am aiming for rapid development of practical applications, through joint research, including prospective research, with clinical researchers.

Conflict of Interest: K. H. has filed a patents for ACTN4 biomarkers.

References

1. Palmer TD, Ashby WJ, Lewis JD, Zijlstra A. Targeting tumor cell motility to prevent metastasis. Adv Drug Deliv Rev. 2011;63(8):568−81. doi: 10.1016/j.addr.2011.04.008. PubMed PMID: 21664937; PubMed Central PMCID: PMC PMC3132821.
2. Yano T, Okamoto T, Fukuyama S, Maehara Y. Therapeutic strategy for postoperative recurrence in patients with non-small cell lung cancer. World J Clin Oncol. 2014;5(5): 1048−54. doi: 10.5306/wjco.v5.i5.1048. PubMed PMID: 254 93240; PubMed Central PMCID: PMCPMC4259931.
3. Honda K. The biological role of actinin-4 (ACTN4) in malignant phenotypes of cancer. Cell Biosci. 2015;5:41. doi: 10.1186/s13578-015-0031-0. PubMed PMID: 26288717; PubMed Central PMCID: PMCPMC4539665.
4. Honda K, Yamada T, Endo R, et al. Actinin-4, a novel actin-bundling protein associated with cell motility and cancer invasion. J Cell Biol. 1998;140(6):1383−93. doi: 10.1083/jcb.140.6.1383. PubMed PMID: 9508771; PubMed Central PMCID: PMCPMC2132673.
5. Otey CA, Carpen O. Alpha-actinin revisited: a fresh look at an old player. Cell Motil Cytoskeleton. 2004;58(2):104− 11. doi: 10.1002/cm.20007. PubMed PMID: 15083532.
6. Honda K, Yamada T, Hayashida Y, et al. Actinin-4 increases cell motility and promotes lymph node metastasis of colorectal cancer. Gastroenterology. 2005;128(1):51−62. PubMed PMID: 15633123.
7. Hayashida Y, Honda K, Idogawa M, et al. E-cadherin regulates the association between beta-catenin and actinin-4. Cancer Res. 2005;65(19):8836−45. doi: 10.1158/ 0008-5472.CAN-05-0718. PubMed PMID: 1620405.
8. Noro R, Honda K, Tsuta K, et al. Distinct outcome of stage I lung adenocarcinoma with ACTN4 cell motility gene amplification. Ann Oncol. 2013;24(10):2594−600. doi: 10.1093/annonc/mdt293. PubMed PMID: 23899839.
9. Miyanaga A, Honda K, Tsuta K, et al. Diagnostic and prognostic significance of the alternatively spliced ACTN 4 variant in high-grade neuroendocrine pulmonary tumours. Ann Oncol. 2013;24(1):84−90. doi: 10.1093/annonc/mds215. PubMed PMID: 22887464.
10. Miura N, Kamita M, Kakuya T, et al. Efficacy of adjuvant chemotherapy for non-small cell lung cancer assessed by metastatic potential associated with ACTN4. Oncotarget. 2016;7(22):33165−78. doi: 10.18632/oncotarget.8890. PubMed PMID: 27121206; PubMed Central PMCID: PMCPM C5078083.
11. Noro R, Ishigame T, Walsh N, et al. A two-gene prognostic classifier for early-stage lung squamous cell carcinoma in multiple large-scale and geographically diverse cohorts. J Thorac Oncol. 2017;12(1):65−76. doi: 10.1016/j.jtho.2016.08.141. PubMed PMID: 27613525; PubMed Central PMCID: PMCPMC6503970.
12. Sugano T, Yoshida M, Masuda M, et al. Prognostic impact of ACTN4 gene copy number alteration in hormone receptor-positive, HER2-negative, node-negative invasive breast carcinoma. Br J Cancer. 2020;122(12):1811−7. doi: 10.1038/s41416-020-0821-y. PubMed PMID: 32265507; PubMed Central PMCID: PMCPMC7283275.
13. Yamamoto S, Tsuda H, Honda K, et al. ACTN4 gene amplification and actinin-4 protein overexpression drive tumour development and histological progression in a high-grade subset of ovarian clear-cell adenocarcinomas. Histopathology. 2012;60(7):1073−83. doi: 10.1111/j.1365-2559.2011.04163.x. PubMed PMID: 22348389.
14. Yamamoto S, Tsuda H, Honda K, et al. Actinin-4 gene amplification in ovarian cancer: a candidate oncogene associated with poor patient prognosis and tumor chemoresistance. Mod Pathol. 2009;22(4):499−507. doi: 10.1038/ modpathol.2008.234. PubMed PMID: 19151661.
15. Yamamoto S, Tsuda H, Honda K, et al. Actinin-4 expression in ovarian cancer: a novel prognostic indicator independent of clinical stage and histological type. Mod Pathol. 2007;20(12):1278−85. doi: 10.1038/modpathol.3800966. PubMed PMID: 17873890.
16. Kikuchi S, Honda K, Tsuda H, et al. Expression and gene amplification of actinin-4 in invasive ductal carcinoma of the pancreas. Clin Cancer Res. 2008;14(17):5348−56. doi: 10.1158/1078-0432.CCR-08-0075. PubMed PMID: 1876552 6.
17. Watanabe T, Ueno H, Watabe Y, et al. ACTN4 copy number increase as a predictive biomarker for chemoradiotherapy of locally advanced pancreatic cancer. Br J Cancer. 2015;112(4):704−13. doi: 10.1038/bjc.2014.623. PubMed PMID: 25602965; PubMed Central PMCID: PMCPMC4333 489.
18. Tanaka N, Yamashita T, Yamamoto S, et al. Histological growth pattern of and alpha-actinin-4 expression in thyroid cancer. Anticancer Res. 2014;34(6):3157−63. PubMed PMID: 24922688.
19. Watabe Y, Mori T, Yoshimoto S, et al. Copy number increase of ACTN4 is a prognostic indicator in salivary gland carcinoma. Cancer Med. 2014;3(3):613−22. doi: 10.1002/cam4.214. PubMed PMID: 24574362; PubMed Central PMCID: PMCPMC4101752.
20. Kakuya T, Mori T, Yoshimoto S, et al. Prognostic significance of gene amplification of ACTN4 in stage I and II oral tongue cancer. Int J Oral Maxillofac Surg. 2017;46(8): 968−76. doi: 10.1016/j.ijom.2017.03.001. PubMed PMID: 28 385383.
21. Fukushima S, Yoshida A, Honda K, et al. Immunohistochemical actinin-4 expression in infiltrating gliomas: association with WHO grade and differentiation. Brain Tumor Pathol. 2014;31(1):11−6. doi: 10.1007/s10014-013-0139-z. PubMed PMID: 23504125.
22. Henry WI, Dubois J, Quick QA. The microtubule inhibiting agent epothilone B antagonizes glioma cell motility associated with reorganization of the actin-binding protein alpha-actinin 4. Oncol Rep. 2011;25(3):887−93. doi: 10.3892/or.2011.1145. PubMed PMID: 21234524.

23. Quick Q, Skalli O. Alpha-actinin 1 and alpha-actinin 4: contrasting roles in the survival, motility, and RhoA signaling of astrocytoma cells. Exp Cell Res. 2010;316(7): 1137–47. doi: 10.1016/j.yexcr.2010.02.011. PubMed PMID: 20156433.
24. Yamada S, Yanamoto S, Yoshida H, et al. RNAi-mediated down-regulation of alpha-actinin-4 decreases invasion potential in oral squamous cell carcinoma. Int J Oral Maxillofac Surg. 2010;39(1):61–7. doi: 10.1016/j.ijom.2009.10.003. PubMed PMID: 19913389.
25. Berania I, Cardin GB, Clement I, et al. Four PTEN-targeting co-expressed miRNAs and ACTN4- targeting miR-548b are independent prognostic biomarkers in human squamous cell carcinoma of the oral tongue. Int J Cancer. 2017;141(11):2318–28. doi: 10.1002/ijc.30915. PubMed PMID: 28779483.
26. Yamagata N, Shyr Y, Yanagisawa K, et al. A training-testing approach to the molecular classification of resected non-small cell lung cancer. Clin Cancer Res. 2003;9 (13):4695–704. PubMed PMID: 14581339.
27. Chen YY, Wang LW, Wang SY, et al. Meta-analysis of postoperative adjuvant chemotherapy without radiotherapy in early stage non-small cell lung cancer. Onco Targets Ther. 2015;8:2033–43. doi: 10.2147/OTT.S88700. PubMed PMID: 26346974; PubMed Central PMCID: PMCPM C4531011.
28. Wong KM, Ding K, Li S, et al. A cost-effectiveness analysis of using the JBR.10-based 15-gene expression signature to guide adjuvant chemotherapy in early stage non-small-cell lung cancer. Clin Lung Cancer. 2017;18(1):e41–7. doi: 10.1016/j.cllc.2016.06.009. PubMed PMID: 27502323.
29. Hsu KS, Kao HY. Alpha-actinin 4 and tumorigenesis of breast cancer. Vitam Horm. 2013;93:323–51. doi: 10.1016/B 978-0-12-416673-8.00005-8. PubMed PMID: 23810014; PubMed Central PMCID: PMC4143506.
30. Chakraborty S, Reineke EL, Lam M, et al. Alpha-actinin 4 potentiates myocyte enhancer factor-2 transcription activity by antagonizing histone deacetylase 7. J Biol Chem. 2006;281(46):35070–80. doi: 10.1074/jbc.M602474200. PubMed PMID: 16980305.
31. Khurana S, Chakraborty S, Cheng X, Su YT, Kao HY. The actin-binding protein, actinin alpha 4 (ACTN4), is a nuclear receptor coactivator that promotes proliferation of MCF-7 breast cancer cells. J Biol Chem. 2011;286(3):1850–9. doi: 10.1074/jbc.M110.162107. PubMed PMID: 21078666; PubMed Central PMCID: PMC3023480.
32. Babakov VN, Petukhova OA, Turoverova LV, et al. RelA/NF-kappaB transcription factor associates with alpha-actinin-4. Exp Cell Res. 2008;314(5):1030–8. doi: 10.1016/j.yexcr.2007.12.001. PubMed PMID: 18215660.
33. Liu X, Chu KM. alpha-Actinin-4 promotes metastasis in gastric cancer. Lab Invest. 2017;97(9):1084–94. doi: 10.1038/labinvest.2017.28. PubMed PMID: 28581489.
34. Welsch T, Keleg S, Bergmann F, Bauer S, Hinz U, Schmidt J. Actinin-4 expression in primary and metastasized pancreatic ductal adenocarcinoma. Pancreas. 2009;38(8):968–76. doi: 10.1097/MPA.0b013e3181b28d6f. PubMed PMID: 19672209.
35. Cho WC, Shin YK, Na YS, Ryu MH, Ku JL, Kang YK. The role of novel fusion genes in human GIST cell lines derived from imatinib-resistant GIST patients: A therapeutic potential of fusion gene. Biochem Biophys Res Commun. 2020;529(3):699–706. doi: 10.1016/j.bbrc.2020.05.174. PubMed PMID: 32736695.
36. Jung J, Kim S, An HT, Ko J. alpha-Actinin-4 regulates cancer stem cell properties and chemoresistance in cervical cancer. Carcinogenesis. 2020;41(7):940–9. doi: 10.1093/carcin/bgz168. PubMed PMID: 31584624.
37. Nowee ME, Snijders AM, Rockx DA, et al. DNA profiling of primary serous ovarian and fallopian tube carcinomas with array comparative genomic hybridization and multiplex ligation-dependent probe amplification. J Pathol. 2007;213(1):46–55. doi: 10.1002/path.2217. PubMed PMID: 17668415.
38. Jasavala R, Martinez H, Thumar J, et al. Identification of putative androgen receptor interaction protein modules: cytoskeleton and endosomes modulate androgen receptor signaling in prostate cancer cells. Mol Cell Proteomics. 2007;6(2):252–71. doi: 10.1074/mcp.M600169-MCP200. PubMed PMID: 17052974.
39. Ishizuya Y, Uemura M, Narumi R, et al. The role of actinin-4 (ACTN4) in exosomes as a potential novel therapeutic target in castration-resistant prostate cancer. Biochem Biophys Res Commun. 2020;523(3):588–94. doi: 10.1016/j.bbrc.2019.12.084. PubMed PMID: 31941606.
40. Shao H, Li S, Watkins SC, Wells A. alpha-Actinin-4 is required for amoeboid-type invasiveness of melanoma cells. J Biol Chem. 2014;289(47):32717–28. doi: 10.1074/jbc.M 114.579185. PubMed PMID: 25296750; PubMed Central PMCID: PMC4239623.
41. Zhao X, Hsu KS, Lim JH, Bruggeman LA, Kao HY. alpha-Actinin 4 potentiates nuclear factor kappa-light-chain-enhancer of activated B-cell (NF-kappaB) activity in podocytes independent of its cytoplasmic actin binding function. J Biol Chem. 2015;290(1):338–49. doi: 10.1074/jbc.M114.597260. PubMed PMID: 25411248; PubMed Central PMCID: PMC4281736.
42. Burmeister T, Meyer C, Schwartz S, et al. The MLL recombinome of adult CD10-negative B-cell precursor acute lymphoblastic leukemia: results from the GMALL study group. Blood. 2009;113(17):4011–5. doi: 10.1182/blood-2008-10-183483. PubMed PMID: 19144982.
43. Yang JJ, Park TS, Lee ST, et al. Molecular characterization and clinical course of MLL-ACTN4 rearrangement in therapy-related hematologic malignancies. Haematologica. 2014;99(4):e49–51. doi: 10.3324/haematol.2013.102798. PubMed PMID: 24561789; PubMed Central PMCID: PMC 3971093.
44. Agarwal N, Adhikari AS, Iyer SV, Hekmatdoost K, Welch DR, Iwakuma T. MTBP suppresses cell migration and filopodia formation by inhibiting ACTN4. Oncogene. 2013;32(4):462–70. doi: 10.1038/onc.2012.69. PubMed PMID: 22370640; PubMed Central PMCID: PMC3742333.
45. Iwakuma T, Agarwal N. MDM2 binding protein, a novel metastasis suppressor. Cancer Metastasis Rev. 2012;31(3-4):633–40. doi: 10.1007/s10555-012-9364-x. PubMed PMID: 22684342.
46. Kaplan JM, Kim SH, North KN, et al. Mutations in ACTN 4, encoding alpha-actinin-4, cause familial focal segmental glomerulosclerosis. Nat Genet. 2000;24(3):251–6. doi: 10.1038/73456. PubMed PMID: 10700177.
47. Kaplan J, Pollak MR. Familial focal segmental glomerulosclerosis. Curr Opin Nephrol Hypertens. 2001;10(2):183–7. PubMed PMID: 11224692.
48. Kato H, Ichinose Y, Ohta M, et al. A randomized trial of adjuvant chemotherapy with uracil-tegafur for adenocarcinoma of the lung. N Engl J Med. 2004;350(17):1713–21. doi: 10.1056/NEJMoa032792. PubMed PMID: 15102997.
49. Hamada C, Tanaka F, Ohta M, et al. Meta-analysis of postoperative adjuvant chemotherapy with tegafur-uracil in non-small-cell lung cancer. J Clin Oncol. 2005;23(22): 4999–5006. doi: 10.1200/JCO.2005.09.017. PubMed PMID:

16051951.
50. Hamada C, Tsuboi M, Ohta M, et al. Effect of postoperative adjuvant chemotherapy with tegafur-uracil on survival in patients with stage IA non-small cell lung cancer: an exploratory analysis from a meta-analysis of six randomized controlled trials. J Thorac Oncol. 2009;4(12): 1511−6. doi: 10.1097/JTO.0b013e3181bbf1f2. PubMed PMID: 19875974.
51. Butts CA, Ding K, Seymour L, et al. Randomized phase III trial of vinorelbine plus cisplatin compared with observation in completely resected stage IB and II non-small-cell lung cancer: updated survival analysis of JBR-10. J Clin Oncol. 2010;28(1):29−34. doi: 10.1200/JCO.2009.24.0333. PubMed PMID: 19933915; PubMed Central PMCID: PMCPMC2799232.
52. Zhu CQ, Ding K, Strumpf D, et al. Prognostic and predictive gene signature for adjuvant chemotherapy in resected non-small-cell lung cancer. J Clin Oncol. 2010;28 (29):4417−24. doi: 10.1200/JCO.2009.26.4325. PubMed PMID: 20823422; PubMed Central PMCID: PMCPMC2988 634.
53. D'Cruz AK, Vaish R, Kapre N, et al. Elective versus therapeutic neck dissection in node-negative oral cancer. N Engl J Med. 2015;373(6):521−9. doi: 10.1056/NEJMoa 1506007. PubMed PMID: 26027881.
54. Tanaka K, Hanai N, Eba J, et al. Randomized phase III study to evaluate the value of omission of prophylactic neck dissection for stage I/II tongue cancer: Japan Clinical Oncology Group study (JCOG1601, RESPOND). Jpn J Clin Oncol. 2018;48(12):1105−8. doi: 10.1093/jjco/hyy125. PubMed PMID: 30346569.

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