Biphasic α2β1 and Breast Cancer

Subjects: Oncology

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Integrins participate in the pathogenesis and progression of tumors at many stages during the metastatic cascade. However, current evidence for the role of integrins in breast cancer progression is contradictory and seems to be dependent on tumor stage, differentiation status, and microenvironmental influences. While some studies suggest that loss of α2β1 enhances cancer metastasis, other studies suggest that this integrin is pro-tumorigenic. However, few studies have looked at α2β1 in the context of bone metastasis. In this study, we aimed to understand the role of α2β1 integrin in breast cancer metastasis to bone. To address this, we utilized in vivo models of breast cancer metastasis to bone using MDA-MB-231 cells transfected with an α2 expression plasmid (MDA-OEα2). MDA cells overexpressing the α2 integrin subunit had increased primary tumor growth and dissemination to bone but had no change in tumor establishment and bone destruction. Further in vitro analysis revealed that tumors in the bone have decreased α2β1 expression and increased osteolytic signaling compared to primary tumors. Taken together, these data suggest an inverse correlation between α2β1 expression and bone-metastatic potential. Inhibiting α2β1 expression may be beneficial to limit the expansion of primary tumors but could be harmful once tumors have established in bone.

tumor-induced bone disease breast cancer bone metastasis integrin α2β1

1. Introduction

Breast cancer is the most common cancer among women and the second leading cause of cancer-related deaths [1]. Despite advances in early detection and therapeutic options for patients, metastatic disease remains the leading cause of patient mortality. Metastatic breast cancer cells have a high preference for bone. It is estimated that 70% of patients with metastatic disease will have bone involvement [2][3], resulting in increased fracture risk, hypercalcemia, increased morbidity and decreased quality of life [4]. Despite the high prevalence of bone metastases, the pathology and risk factors of breast cancer metastasis to bone are not fully understood.

Recent studies have revealed that the expression profile of primary tumors and composition of the surrounding extracellular matrix (ECM) are important factors contributing to tumor progression and metastasis [5][6][7]. Specifically, the expression of cell surface adhesion receptors, such as integrins, have been shown to be prognostic [8][9][10]. Integrins are αβ heterodimeric transmembrane receptors that support cell adhesion to the extracellular matrix (ECM) and trigger intracellular signaling that can modify cellular behavior [11][12]. Alterations in integrin expression are commonly found in cancer and have been linked to increased tumor proliferation, invasion, and secondary site colonization, as well as decreased patient survival [13][14].

α2β1 integrin has been implicated as an important target in cancer progression due to its critical role in a variety of cancers [15]. Studies have shown that α2β1 integrin is a marker of malignant progression in prostate cancer [16][17][18], liver cancer [19][20], gastric cancer [21][22][23], and melanoma [24]. However, in breast cancer, there is conflicting evidence for the role of α2β1 integrin. While some studies suggest that the loss of α2β1 promotes breast cancer metastasis [25][26], other studies suggest that high α2β1 expression correlates with a metastatic phenotype [19][27][28]. It is believed that α2β1 integrin may also play an important role in bone metastasis due to its high affinity to bind collagen type 1 (which is the main component of the organic part of bone) [29]. While α2β1 has been shown to promote skeletal metastases in prostate cancer [18][30], very few studies have looked at this integrin in the context of breast cancer metastasis to bone.

Thus, this study aimed to investigate the role of α2β1 integrin in breast cancer metastasis to bone. We hypothesized that α2β1 integrin promotes a bone-metastatic potential of breast cancer cells. To test this hypothesis, we used in vivo mouse models of cancer metastasis to bone (orthotopic: mammary fat pad—MFP, metastasis and colonization: intracardiac—IC, and establishment in bone: intratibial—IT). In order to investigate tumor progression and metastasis with respect to α2β1 expression, we developed high expressing MDA-MB-231 breast cancer cells by transfecting cells with an α2 DNA plasmid (OE-α2). In this study, we demonstrate that α2β1 integrin promotes tumor development at the primary site and metastasis to bone but has no effect on bone destruction once tumors have established in bone.

2. α2β1 Expression Correlates with an Invasive and Migratory Phenotype

Integrin α2β1 expression is often upregulated in metastatic cancer cells [8][31][32]. Whole exome sequencing of tumor biopsies from patients collected under the Metastatic Breast Cancer Project revealed that metastatic primary tumors have higher ITGA2 and ITGB1 copy number compared to non-metastatic primary tumors (**Figure 1**A). In order to study the effect of elevated α2β1 expression on breast tumor behavior, we generated a model of MDA-MB-231 breast cancer cells with high α2β1 by stably transfecting a bone-derived clone of MDA-MB-231 (MDA-Bone) with an expression plasmid for α2 (MDA-OEα2) or an empty vector control (MDA-Ctrl). Manipulation of integrin expression and signaling was confirmed by qPCR and western blot analysis (**Figure 1**B,C). Although we only introduced an α2 expression plasmid into the cells, we were able to achieve significantly higher mRNA and protein expression for both α2 and β1 subunits compared to Ctrl cells. Downstream integrin signaling was also shown to be activated in MDA-OEα2 cells (**Figure 1**C).

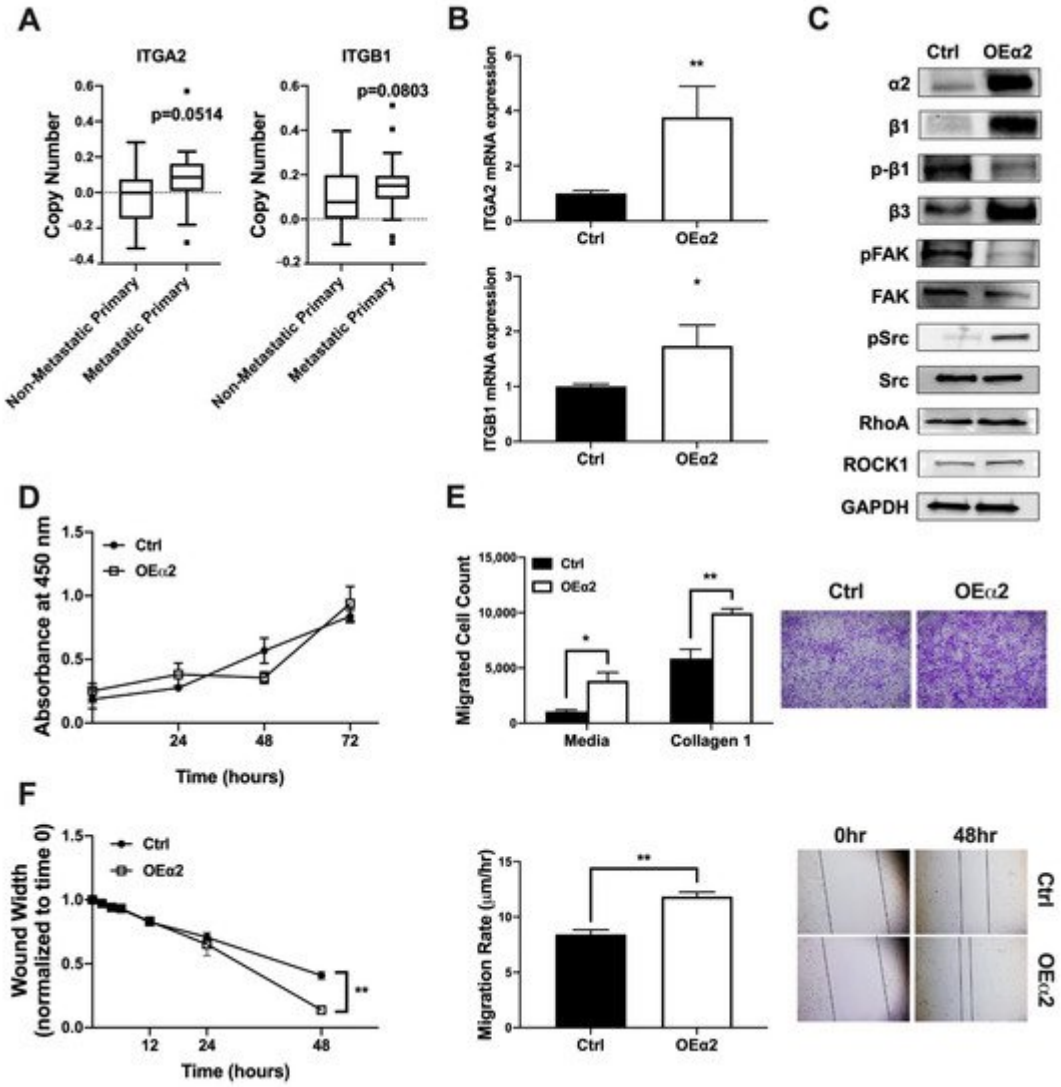

Figure 1. (**A**) Whole exome sequencing of tumor biopsies from patients collected under the Metastatic Breast Cancer Project was analyzed for copy number alterations in ITGA2 and ITGB1. N = 14 non-metastatic primary, N = 42 metastatic primary tumors. Mann–Whitney test. (**B**) qPCR and (**C**) western blot analysis confirmed that cells overexpressing α2 (OEα2) had increased ITGA2 and ITGB1 mRNA expression as well as increased α2 and β1 subunit protein expression and activated integrin signaling. (**D**) An MTS proliferation assay showed no significant difference in cell growth at 24, 72, or 120 h in cells expressing high α2 compared to control. (**E**) A significantly higher number of MDA-OEα2 tumor cells compared to MDA-Ctrl cells migrated in a Transwell Invasion Assay using either complete media or media + Collagen 1 as a chemoattractant. (**F**) MDA-OEα2 cells migrated at a faster rate compared to MDA-Ctrl cells in a scratch assay as measured by changes in wound width over time. N = 3 biological replicates. Data presented as fold change over control (Ctrl). Student's t-test * $p < 0.05$, ** $p < 0.01$.

We further wanted to analyze these cells for changes in proliferation and invasive or migratory phenotype in response to enhanced integrin expression. While there was no change in tumor proliferation, we found that tumor cells with high α2β1 expression were more invasive and migratory (**Figure 1**D,F). A higher number of MDA-OEα2 cells migrated in a transwell invasion assay compared to MDA-Ctrl cells. Furthermore, a scratch assay revealed an increased migration rate in MDA-OEα2 cells.

3. α2β1 Integrin Promotes Primary Tumor Growth and Dissemination to Bone

Current evidence suggests that α2β1 integrin can act as both a tumor suppressor [25][26][33] and a tumor promoter [19][27][28] in breast cancer and seems to be dependent on tumor status [34]. While most of these studies have looked at invasion and dissemination to soft tissue sites, few studies have elucidated the role of α2β1 integrin in breast cancer dissemination to the bone. Here, we used an in vivo mammary fat pad model of human breast cancer to investigate the effect of elevated α2β1 expression on primary tumor growth, flow cytometry analysis to determine changes in circulating tumor cells (CTCs) and dissemination to bone, and histology analysis to investigate metastases to the lung or bone.

Tumor growth analysis revealed that breast cancer cells expressing high levels of α2β1 have increased growth in vivo (**Figure 2**A) and larger tumors at sacrifice (**Figure 2**B,C) compared to control tumors. Immunohistochemical analysis for the α2 integrin subunit confirmed higher expression in MDA-OEα2 tumors. Interestingly, we found that α2 expression in the control tumors was higher at the periphery of the tumor, while α2 overexpressing tumors had high expression throughout the tumor (Supplemental Figure S1). These data support our hypothesis that α2β1 is needed for migration and invasion from the primary site.

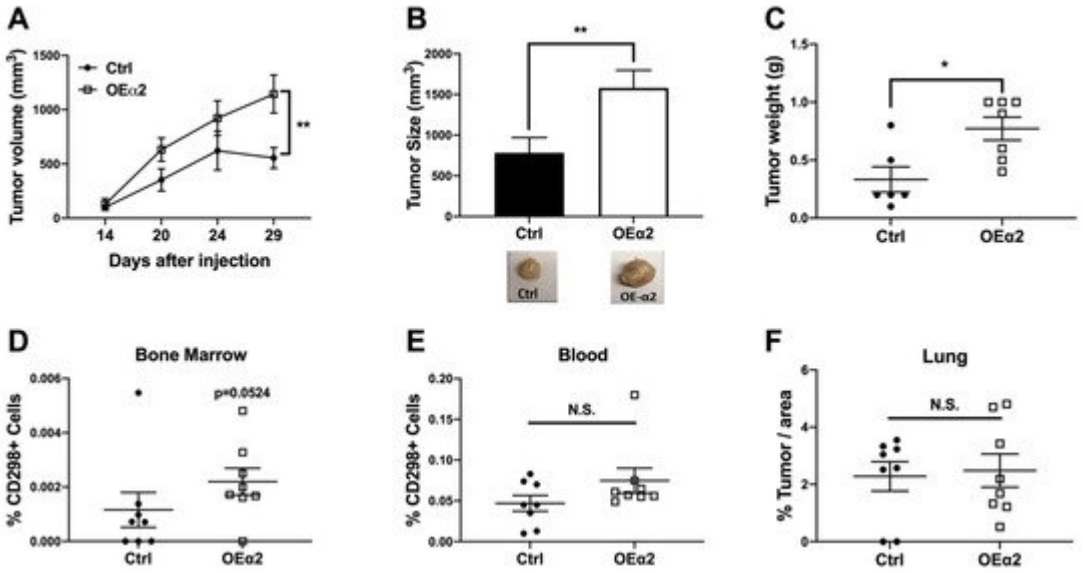

Figure 2. Four–six-week-old athymic nude mice were injected with 5 × 10^5 MDA-Ctrl or MDA-OEα2 cells into the mammary fat pad. Mice injected with MDA cells overexpressing α2 had increased tumor growth (**A**), larger tumors at sacrifice (**B**), and increased tumor weight (**C**) compared to mice injected with control MDAs. (**D**,**E**) Flow cytometry analysis was performed on bone marrow and blood using a novel protocol to detect the presence of human tumor cells using the marker CD298 (ATP1B3). Mice injected with cells overexpressing the integrin α2 subunit had an increased % $CD298^+$ cells in the bone marrow (**D**), and a trending (n.s.) increase in the blood (**E**) compared to mice injected with control cells. (**F**) Lung metastases were quantified by histological analysis, and no difference was observed between OEα2 and Ctrl cells. N = 8 per group. Mice were sacrificed 30 days post tumor inoculation. Two-way ANOVA and Mann–Whitney test. * $p < 0.05$, ** $p < 0.01$.

Using a novel flow cytometry technique for detecting disseminated tumor cells in models of low tumor burden using the human cell marker CD298 [35], we analyzed plasma for the presence of CTCs and bone marrow for the presence of disseminated tumor cells (DTCs) (gating scheme can be found in Supplemental Figure S2). Consistent with the tumor growth analysis, we found that mice injected with MDA-OEα2 cells had an increase in the number of disseminated tumor cells in the bone marrow compared to mice injected with MDA-Ctrl cells (**Figure 2**D). The presence of tumor cells in the bone marrow was confirmed by histomorphometry analysis with H&E staining (Supplemental Figure S3). Although not statistically significant, there was a trending increase in the presence of CTCs for mice given MDA-OEα2 cells (**Figure 2**E). While there was an observed increase in bone metastases (Supplemental Figure S3), histological analysis revealed no significant difference in lung metastases (**Figure 2**F). Taken together, these data reveal that high α2 expression in tumors at the primary site results in increased tumor growth and increased dissemination to the bone. Higher α2 expression at the periphery of the tumor and the presence of CTCs also suggest that α2β1 integrin may be creating a more invasive and metastatic phenotype in these breast cancer cells.

4. Breast Cancer Cells with High α2β1 Expression Have Increased Colonization in the Bone, but Have No Effect on Bone Destruction

Integrins have been shown to play an important role at many stages of the metastatic cascade [13]. Specifically, β1 integrins have been implicated in extravasation from the vasculature and colonization into secondary sites [13][32][36]. To study the role of α2β1 integrin in tumor cell colonization of the bone, we used an in vivo metastasis model where tumor cells are introduced directly into the vasculature via intracardiac (IC) injection. Four–six-week-old female athymic nude mice were injected with MDA-OEα2 or -Ctrl cells. Histological analysis revealed that high expression of α2β1 integrin on the surface of tumor cells increased the amount of tumor cells that colonized the bone but had no effect on subsequent bone destruction (**Figure 3**A–C). There was significantly higher % tumor area in the tibias of mice given MDA-OEα2 cells compared to mice given MDA-Ctrl cells, but no significant differences were found in bone volume (%BV/TV) by μCT or lesion area by X-ray. These data support our findings in the MFP model that α2β1 expression correlates with an increase in breast tumor dissemination to bone.

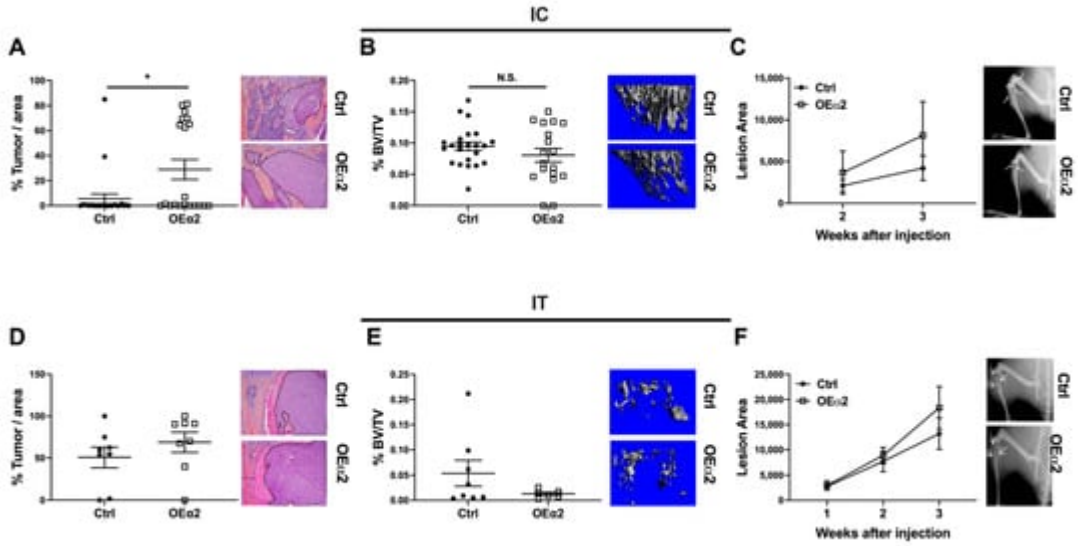

Figure 3. (**A**–**C**) Four–six-week-old athymic nude mice were injected via intracardiac (IC) injection with 1 × 10^5 MDA-Ctrl or MDA-OEα2 cells. (**A**) H&E staining revealed increased percentage of tumor cells in the tibias of mice injected with MDA-OEα2 cells compared to mice injected with MDA-Ctrl cells. (**B**) μCT analysis and (**C**) X-ray analysis show no change in bone volume and lesion area. N = 12 mice per group, 2 bones analyzed per mouse. IC mice were sacrificed 30 days post tumor inoculation. Mann–Whitney test. * $p < 0.05$. (**D**–**F**) Four–six-week-old athymic nude mice were injected via intratibial (IT) injection with 1 × 10^5 MDA-Ctrl or MDA-OEα2cells. (**D**) Histomorphometry reveals no difference in tumor area between MDA-OEα2 and MDA-Ctrl injected mice. (**E**) μCT analysis shows no difference in bone volume, and (**F**) X-ray analysis shows no difference in the lesion area. N = 8 per group. IT mice were sacrificed 21 days post tumor inoculation. Mann–Whitney test.

To study the effect of α2β1 expression on tumors that have already established in bone, we used an in vivo model of tumor growth in bone. MDA-OEα2 or MDA-Ctrl cells were injected in the right tibia (IT injection) of 4–6-week-old athymic nude mice. PBS was injected into the contralateral limb for a non-tumor control. Interestingly, high α2 expression in established bone metastases had no effect on overall tumor burden and bone destruction (**Figure 3**D–F). Substantial bone destruction was observed by X-ray and μCT analysis for mice given MDA-Ctrl cells and for mice given MDA-OEα2 cells. H&E staining showed significant tumor burden in the tibias of both sets of mice, with no significant difference in % tumor area between OEα2 and Ctrl cells.

5. Osteolytic Breast Tumor Cells Have Decreased α2β1 Expression

The in vivo data reveals α2β1 to be a tumor promoter at earlier stages of metastasis, such as invasion and extravasation, but seem to have no effect on tumors already established in bone. To further understand the phenotype, we wanted to evaluate differences in gene expression for tumors that metastasize to bone and cause bone destruction versus primary tumors. We analyzed the mRNA and protein expression profiles of our bone-metastatic clone of MDA-MB-231 cells (MDA-Bone) and the parental MDA-MB-231 cells from ATCC (MDA-Parental) and found that bone-metastatic cells have decreased integrin signaling (**Figure 4**A,B). MDA-Bone cells

have decreased expression of α2 and β1 subunits and decreased protein expression of the downstream signaling factors SRC, RhoGTP, and ROCK. Due to its critical role in bone metastases [37], the integrin subunit β3 was also evaluated; however, there was no significant difference in β3 mRNA or protein expression, suggesting that these changes in integrin signaling are driven primarily by α2β1.

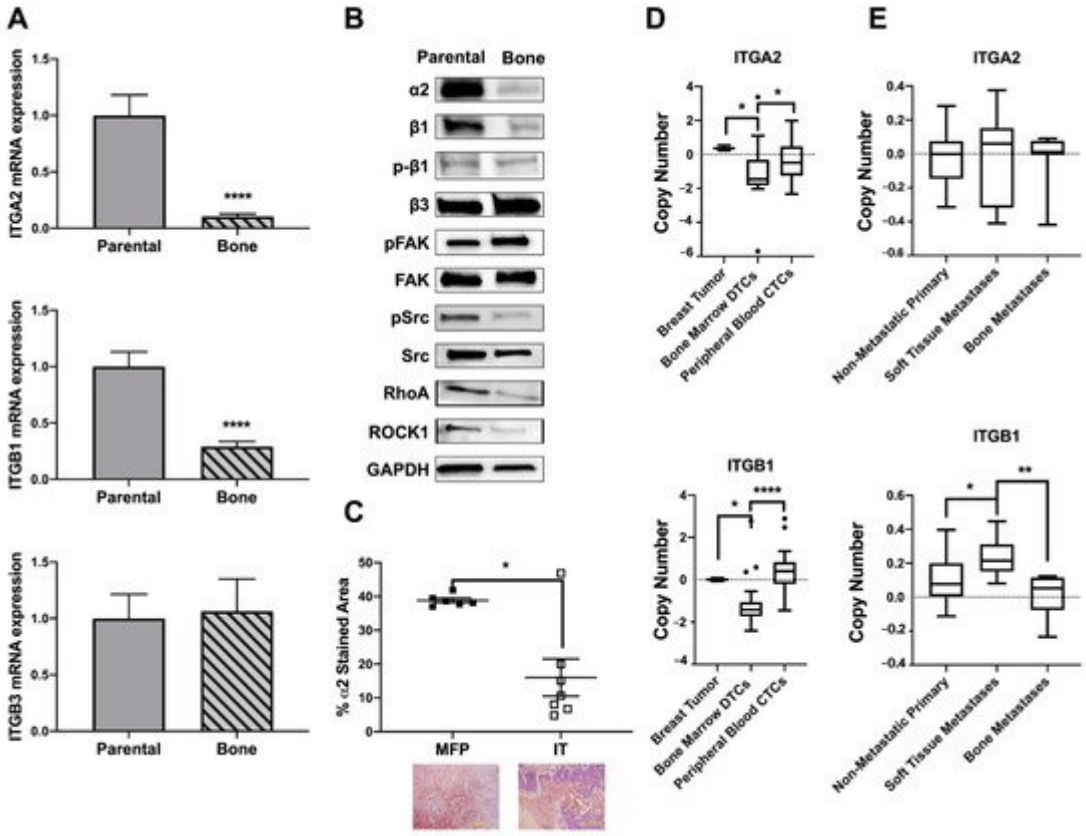

Figure 4. (**A**,**B**) A bone metastatic clone of MDA-MB-231 (Bone) and the parental MDA-MB-231 cells (Parental) were analyzed for integrin expression by (**A**) qPCR and (**B**) western blot analysis. Bone metastatic cells have less expression of the integrin subunits α2 and β1 and downstream integrin signaling compared to parental cells. Data presented as fold change over parental. N = 3 biological replicates. Student's t-test. **** $p < 0.0001$. (**C**) α2 expression was analyzed in vivo by immunohistochemistry revealing that tumors in the bone (IT, intratibial injection) have less tumor expression of α2 compared to tumors in the primary site (MFP, mammary fat pad injection). N = 8 mice per group. Mann–Whitney test. * $p < 0.05$. (**D**) Microarray database analysis collected from the NCBI gene expression omnibus GEO accession GSE27574 revealed that disseminated tumor cells (DTCs) in the bone marrow have fewer copy numbers of ITGA2 and ITGB1 compared to primary breast tumors and circulating tumor cells (CTCs). N = 3 breast tumors, N = 24 DTCs, N = 28 CTCs. Kruskal–Wallis test. * $p < 0.05$, **** $p < 0.0001$. (**E**) Whole exome sequencing of tumor biopsies from patients collected under the Metastatic Breast Cancer Project was analyzed for copy number alterations in ITGA2 and ITGB1. Soft tissue biopsies had higher ITGB1 than non-metastatic primary tumors and tumor biopsies from bone metastases had fewer copy numbers of ITGB1 compared to soft tissue metastases. No significant difference was observed for ITGA2. N = 14 non-metastatic primary, N = 42 metastatic primary tumors, N = 8 bone metastases, N = 10 soft tissue metastases. Kruskal–Wallis test. * $p < 0.05$, ** $p < 0.01$.

This decrease in α2β1 integrin expression in bone metastases was also observed in vivo. Immunohistochemical analysis revealed that tumors in the bone have significantly lower expression of α2 compared to tumors in the mammary fat pad (**Figure 4**C). Publicly available genome expression datasets of metastatic breast cancer patients were analyzed to confirm the clinical relevance of our findings. Single-cell microarray analysis of circulating tumor cells (CTCs) isolated from peripheral blood and disseminated tumor cells (DTCs) isolated from bone marrow aspirates of breast cancer patients [38] (GEO accession GSE27574) reveals that DTCs have a decrease in copy number of ITGA2 and ITGB1 compared to primary breast tumor samples and CTCs (**Figure 4**D). Whole exome sequencing from the Metastatic Breast Cancer Project [39][40] was analyzed for putative copy number alterations of ITGA2 and ITGB1 in biopsies of primary tumors with no evidence of metastatic disease (non-metastatic primary), biopsies of bone metastases, and biopsies of soft tissue metastases (**Figure 4**E). While no significant difference was observed for ITGA2, ITGB1 was significantly increased in soft tissue metastases, compared to primary tumors, and bone metastases had lower ITGB1 than soft tissue metastases.

6. α2β1 Integrin Expression Is Inversely Correlated with Osteolytic Gene Expression

It is well documented that once tumors metastasize to bone, they can respond to stimuli from the bone microenvironment to adapt a bone-destructive phenotype [41][42]. Once in the bone, breast tumors begin to secrete parathyroid hormone-related protein (PTHrP) to stimulate osteoclastogenesis and bone destruction [43][44][45]. This increased bone destruction causes the release of matrix-derived proteins such as transformation growth factor β (TGF-β), which then feeds back on the tumor cells to promote further production of PTHrP [46][47], which is regulated by the transcription factor Gli2 [48][49]. To evaluate the expression patterns of genes involved in tumor-induced osteolysis with respect to α2β1 integrin, we performed qPCR and western blot analysis for PTHrP and Gli2 (**Figure 5**A,B) and TGFβrII and RUNX2 (Supplemental Figure S4) in MDA-Ctrl or MDA-OEα2 cells, and MDA-Parental or MDA-Bone cells. MDA-MB-231 cells overexpressing α2 integrin had decreased PTHrP and Gli2 expression compared to bone-metastatic cells and Ctrl cells, while no significant change was observed for TGFβrII and RUNX2. Comprehensive RNA sequencing data collected as a part of the MET500 cohort [50] was analyzed for gene expression of Gli2, PTHLH, ITGB1, and ITGA2 in metastatic breast cancer samples. Spearman correlation analysis of gene signatures in metastatic biopsies of breast cancer reveal a significant ($p < 0.001$) negative correlation between PTHLH and ITGA2 ($p < 0.001$), PTHLH and ITGB1 ($p < 0.01$), Gli2 and ITGA2 ($p < 0.001$), and Gli2 and ITGB1 ($p < 0.0001$) (**Figure 5**C,D).

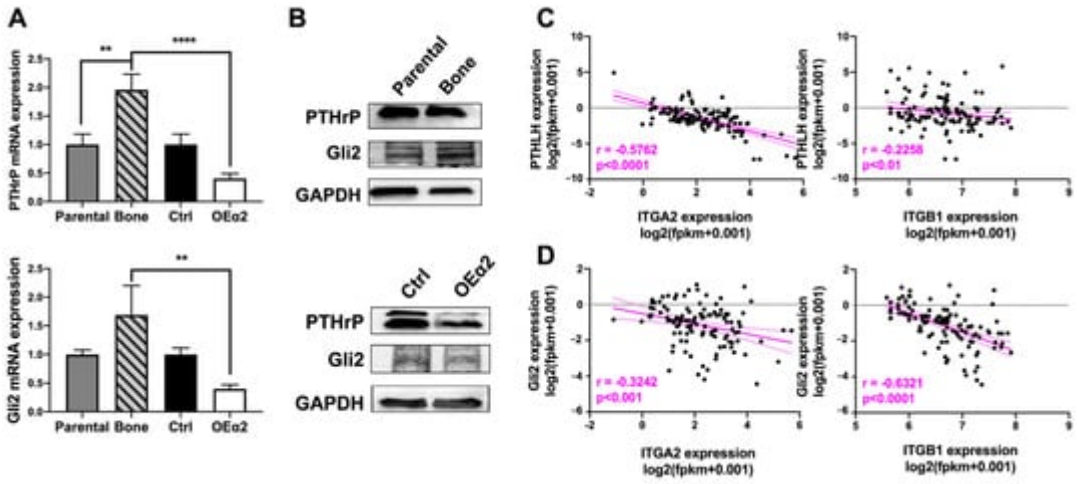

Figure 5. MDA-Parental, MDA-Bone, MDA-Ctrl, and MDA-OEα2 cells were analyzed for osteolytic gene expression by (**A**) qPCR and (**B**) western blot analysis. Tumor cells overexpressing the α2 integrin subunit had decreased PTHrP and Gli2 expression compared to bone and control cells (each set at 1). N = 3 biological replicates. Student's t-test. ** $p < 0.01$, **** $p < 0.0001$. (**C**,**D**) RNA sequencing analysis from metastatic breast cancer biopsies from the MET500 cohort was analyzed for correlation between (**C**) PTHLH, ITGA1, and ITGB1, and (**D**) Gli2, ITGA2, and ITGB1 gene signatures. Spearman correlation analysis reveal a significant negative correlation between integrin α2β1 and osteolytic genes. N = 120 tumor samples.

Taken together, these data support the hypothesis that once tumors metastasize to the bone microenvironment, they undergo genetic changes and adapt a bone destructive phenotype. While expression of α2β1 integrin plays an important role in tumor invasion, extravasation, and dissemination, once tumors establish in bone, they turn off the expression of α2β1 and turn on expression of genes important for growth and survival in bone and bone destruction.

References

1. American Cancer Society. Cancer Facts & Figures; American Cancer Society: Atlanta, GA, USA, 2020.
2. Pulido, C.; Vendrell, I.; Ferreira, A.R.; Casimiro, S.; Mansinho, A.; Alho, I.; Costa, L. Bone metastasis risk factors in breast cancer. Ecancermedicalscience 2017, 11, 715.
3. Buijs, J.T.; van der Pluijm, G. Osteotropic cancers: From primary tumor to bone. Cancer Lett. 2009, 273, 177–193.
4. Coleman, R.E. Clinical Features of Metastatic Bone Disease and Risk of Skeletal Morbidity. Clin. Cancer Res. 2006, 12, 6243s–6249s.

5. Van'T Veer, L.J.; Dai, H.; Van De Vijver, M.J.; He, Y.D.; Hart, A.A.M.; Mao, M.; Peterse, H.L.; Van Der Kooy, K.; Marton, M.J.; Witteveen, A.T.; et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature 2002, 415, 530–536.
6. Chiang, A.C.; Massague, J. Molecular Basis of Metastasis. N. Engl. J. Med. 2008, 359, 2814–2823.
7. Weigelt, B.; Glas, A.; Wessels, L.F.A.; Witteveen, A.T.; Peterse, J.L.; Veer, L.J.V. Gene expression profiles of primary breast tumors maintained in distant metastases. Proc. Natl. Acad. Sci. USA 2003, 100, 15901–15905.
8. Naci, D.; Vuori, K.; Aoudjit, F. Alpha2beta1 integrin in cancer development and chemoresistance. Semin. Cancer Biol. 2015, 35, 145–153.
9. Plantefaber, L.C.; Hynes, R.O. Changes in integrin receptors on oncogenically transformed cells. Cell 1989, 56, 281–290.
10. Hynes, R.O. Integrins: Bidirectional, Allosteric Signaling Machines. Cell 2002, 110, 673–687.
11. Schwartz, M.A.; Schaller, M.D.; Ginsberg, M.H. Integrins: Emerging Paradigms of Signal Transduction. Annu. Rev. Cell Dev. Biol. 1995, 11, 549–599.
12. Shattil, S.J.; Kim, C.; Ginsberg, M.H. The final steps of integrin activation: The end game. Nat. Rev. Mol. Cell Biol. 2010, 11, 288–300.
13. Hamidi, H.; Ivaska, J. Every step of the way: Integrins in cancer progression and metastasis. Nat. Rev. Cancer 2018, 18, 533–548.
14. Albelda, S.M. Role of integrins and other cell adhesion molecules in tumor progression and metastasis—PubMed. Lab. Investig. 1993, 68, 4–17. Available online: (accessed on 7 August 2020).
15. Adorno-Cruz, V.; Liu, H. Regulation and functions of integrin α2 in cell adhesion and disease. Genes Dis. 2019, 6, 16–24.
16. Hall, C.L.; Dai, J.; Van Golen, K.L.; Keller, E.T.; Long, M.W. Type I Collagen Receptor (α2β1) Signaling Promotes the Growth of Human Prostate Cancer Cells within the Bone. Cancer Res. 2006, 66, 8648–8654.
17. Hall, C.L.; Dubyk, C.W.; Riesenberger, T.A.; Shein, D.; Keller, E.T.; van Golen, K.L. Type I Collagen Receptor (α2β1) Signaling Promotes Prostate Cancer Invasion through RhoC GTPase. Neoplasia 2008, 10, 797–803.
18. Sottnik, J.L.; Daignault-Newton, S.; Zhang, X.; Morrissey, C.; Hussain, M.H.; Keller, E.T.; Hall, C.L. Integrin alpha2beta1 (α2β1) promotes prostate cancer skeletal metastasis. Clin. Exp. Metastasis 2013, 30, 569–578.

19. Tabaries, S.; Dong, Z.; Annis, M.G.; Omeroglu, A.; Pepin, F.; Ouellet, V.; Russo, C.; Hassanain, M.; Metrakos, P.; Diaz, Z.H.; et al. Claudin-2 is selectively enriched in and promotes the formation of breast cancer liver metastases through engagement of integrin complexes. Oncogene 2010, 30, 1318–1328.
20. Yoshimura, K.; Meckel, K.F.; Laird, L.S.; Chia, C.Y.; Park, J.-J.; Olino, K.; Tsunedomi, R.; Harada, T.; Iizuka, N.; Hazama, S.; et al. Integrin α2 Mediates Selective Metastasis to the Liver. Cancer Res. 2009, 69, 7320–7328.
21. Si, J.; Li, A.; Zhou, T.; Guo, L. Si Collagen type I regulates β-catenin tyrosine phosphorylation and nuclear translocation to promote migration and proliferation of gastric carcinoma cells. Oncol. Rep. 2010, 23, 1247–1255.
22. Matsuoka, T.; Yashiro, M.; Nishimura, S.; Inoue, T.; Fujihara, T.; Sawada, T.; Kato, Y.; Seki, S.; Chung, K.H.-Y. Increased expression of alpha2beta1-integrin in the peritoneal dissemination of human gastric carcinoma. Int. J. Mol. Med. 2000, 5, 21–26.
23. Lin, M.-T.; Chang, C.-C.; Lin, B.-R.; Yang, H.-Y.; Chu, C.-Y.; Wu, M.-H.; Kuo, M.-L. Elevated Expression of Cyr61 Enhances Peritoneal Dissemination of Gastric Cancer Cells through Integrin α2β1. J. Biol. Chem. 2007, 282, 34594–34604.
24. Moretti, S.; Martini, L.; Berti, E.; Pinzi, C.; Giannotti, B. Adhesion molecule profile and malignancy of melanocytic lesions. Melanoma Res. 1993, 3, 235–239. Available online: (accessed on 7 August 2020).
25. Keely, P.J.; Fong, A.M.; Zutter, M.M.; Santoro, S.A. Alteration of collagen-dependent adhesion, motility, and morphogenesis by the expression of antisense alpha 2 integrin mRNA in mammary cells. J. Cell Sci. 1995, 108 (Pt 2), 595–607.
26. Ramirez, N.E.; Zhang, Z.; Madamanchi, A.; Boyd, K.L.; O'Rear, L.D.; Nashabi, A.; Li, Z.; Dupont, W.D.; Zijlstra, A.; Zutter, M.M. The α2β1 integrin is a metastasis suppressor in mouse models and human cancer. J. Clin. Investig. 2011, 121, 226–237.
27. Dudley, D.T.; Li, X.-Y.; Hu, C.Y.; Kleer, C.G.; Willis, A.L.; Weiss, S.J. A 3D matrix platform for the rapid generation of therapeutic anti-human carcinoma monoclonal antibodies. Proc. Natl. Acad. Sci. USA 2014, 111, 14882–14887.
28. Ibaragi, S.; Shimo, T.; Hassan, N.M.M.; Isowa, S.; Kurio, N.; Mandai, H.; Kodama, S.; Sasaki, A. Induction of MMP-13 expression in bone-metastasizing cancer cells by type I collagen through integrin α1β1 and α2β1-p38 MAPK signaling. Anticancer Res. 2011, 31, 1307–1313.
29. Schneider, J.G.; Amend, S.R.; Weilbaecher, K.N. Integrins and bone metastasis: Integrating tumor cell and stromal cell interactions. Bone 2011, 48, 54–65.
30. Bonkhoff, H.; Stein, U.; Remberger, K. Differential expression of α6 and α2 very late antigen integrins in the normal, hyperplastic, and neoplastic prostate: Simultaneous demonstration of cell

surface receptors and their extracellular ligands☆. Hum. Pathol. 1993, 24, 243–248.

31. Hall, C.L.; Keller, E.T. Analysis of Integrin Alpha2Beta1 (α2β1) Expression as a Biomarker of Skeletal Metastasis. Biomark. Bone Dis. 2017, 487–506.
32. Pan, B.; Guo, J.; Liao, Q.; Zhao, Y. β1 and β3 integrins in breast, prostate and pancreatic cancer: A novel implication (Review). Oncol. Lett. 2018, 15, 5412–5416.
33. Zutter, M.M.; Santoro, S.A.; Staatz, W.D.; Tsung, Y.L. Re-expression of the alpha 2 beta 1 integrin abrogates the malignant phenotype of breast carcinoma cells. Proc. Natl. Acad. Sci. USA 1995, 92, 7411–7415.
34. Zutter, M.M.; Mazoujian, G.; Santoro, S.A. Decreased expression of integrin adhesive protein receptors in adenocarci-noma of the breast. Am. J. Pathol. 1990, 137, 863–870. Available online: (accessed on 7 August 2020).
35. Sowder, M.E.; Johnson, R.W. Enrichment and detection of bone disseminated tumor cells in models of low tumor burden. Sci. Rep. 2018, 8, 14299.
36. Chen, M.B.; Lamar, J.; Li, R.; Hynes, R.O.; Kamm, R.D. Elucidation of the Roles of Tumor Integrin β1 in the Extravasation Stage of the Metastasis Cascade. Cancer Res. 2016, 76, 2513–2524.
37. Kwakwa, K.A.; Sterling, J.A. Integrin αvβ3 Signaling in Tumor-Induced Bone Disease. Cancers 2017, 9, 84.
38. Mathiesen, R.R.; Fjelldal, R.; Liestøl, K.; Due, E.U.; Geigl, J.B.; Riethdorf, S.; Borgen, E.; Rye, I.H.; Schneider, I.J.; Obenauf, A.C.; et al. High-resolution analyses of copy number changes in disseminated tumor cells of patients with breast cancer. Int. J. Cancer 2011, 131, E405–E415.
39. Wagle, N.; Painter, C.; Anastasio, E.; Dunphy, M.; McGillicuddy, M.; Kim, D.; Jain, E.; Buendia-Buendia, J.; Cohen, O.; Knelson, E.; et al. The Metastatic Breast Cancer (MBC) project: Accelerating translational research through direct patient engagement. J. Clin. Oncol. 2017, 35, 1076.
40. The Metastatic Breast Cancer Project. Available online: (accessed on 7 August 2020).
41. Buenrostro, D.; Mulcrone, P.L.; Owens, P.; Sterling, J.A. The Bone Microenvironment: A Fertile Soil for Tumor Growth. Curr. Osteoporos. Rep. 2016, 14, 151–158.
42. Sterling, J.A.; Edwards, J.R.; Martin, T.J.; Mundy, G.R. Advances in the biology of bone metastasis: How the skeleton affects tumor behavior. Bone 2011, 48, 6–15.
43. Powell, G.J.; Southby, J.; Danks, J.A.; Stillwell, R.G.; Hayman, J.A.; Henderson, M.A.; Bennett, R.C.; Martin, T.J. Localization of parathyroid hormone-related protein in breast cancer metastases: Increased incidence in bone compared with other sites. Cancer Res. 1991, 51, 3059–3061.

44. Southby, J.; Kissin, M.W.; Danks, J.A.; Hayman, J.A.; Moseley, J.M.; Henderson, M.A.; Bennett, R.C.; Martin, T.J. Immunohistochemical localization of parathyroid hormone-related protein in human breast cancer. Cancer Res. 1990, 50, 7710–7716.
45. Boyle, W.J.; Simonet, W.S.; Lacey, D.L. Osteoclast differentiation and activation. Nat. Cell Biol. 2003, 423, 337–342.
46. Yin, J.J.; Selander, K.; Chirgwin, J.M.; Dallas, M.; Grubbs, B.G.; Wieser, R.; Massague, J.; Mundy, G.R.; Guise, T.A. TGF-β signaling blockade inhibits PTHrP secretion by breast cancer cells and bone metastases development. J. Clin. Investig. 1999, 103, 197–206.
47. Biswas, S.; Nyman, J.S.; Alvarez, J.; Chakrabarti, A.; Ayres, A.; Sterling, J.; Edwards, J.; Rana, T.; Johnson, R.; Perrien, D.S.; et al. Anti-Transforming Growth Factor ß Antibody Treatment Rescues Bone Loss and Prevents Breast Cancer Metastasis to Bone. PLoS ONE 2011, 6, e27090.
48. Johnson, R.W.; Nguyen, M.P.; Padalecki, S.S.; Grubbs, B.G.; Merkel, A.; Oyajobi, B.O.; Matrisian, L.M.; Mundy, G.R.; Sterling, J.A. TGF-β Promotion of Gli2-Induced Expression of Parathyroid Hormone-Related Protein, an Important Osteolytic Factor in Bone Metastasis, Is Independent of Canonical Hedgehog Signaling. Cancer Res. 2011, 71, 822–831.
49. Sterling, J.A.; Oyajobi, B.O.; Grubbs, B.; Padalecki, S.S.; Munoz, S.A.; Gupta, A.; Story, B.; Zhao, M.; Mundy, G.R. The Hedgehog Signaling Molecule Gli2 Induces Parathyroid Hormone-Related Peptide Expression and Osteolysis in Metastatic Human Breast Cancer Cells. Cancer Res. 2006, 66, 7548–7553.
50. Robinson, D.R.; Wu, Y.-M.; Lonigro, R.J.; Vats, P.; Cobain, E.; Everett, J.; Cao, X.; Rabban, E.; Kumar, C.; Raymond, V.; et al. Integrative clinical genomics of metastatic cancer. Nat. Cell Biol. 2017, 548, 297–303.