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Hypoxia

Inhibiting the phosphatidylinositide 3-kinase pathway blocks radiation-induced metastasis associated with Rho-GTPase and Hypoxia-inducible factor-1 activity

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ABSTRACT

Background and purpose: Undifferentiated follicular and anaplastic thyroid tumours often respond poorly to radiotherapy and show increased metastatic potential. We evaluated radiation-induced effects on metastasis in thyroid carcinoma cells and tumours, mechanistically focusing on phosphatidylinositide 3-kinase (PI3K) and associated pathways.

Material and methods: Migration was analysed in follicular (FTC133) and anaplastic (8505c) cells following radiotherapy (0–6 Gray) with concomitant pharmacological (GDC-0941) or genetic inhibition of PI3K. Hypoxia-inducible factor-1 (HIF-1)-activity was measured using luciferase reporter assays and was inhibited using a dominant-negative variant. Activation and subcellular localisation of target proteins were assessed via Western blot and immunofluorescence. *In vivo* studies used FTC133 xenografts with metastatic lung dissemination assessed *ex vivo*.

Results: Radiation induced migration in a HIF-dependent manner in FTC133 cells but decreased migration in 8505c's. Post-radiation HIF-activity correlated with migratory phenotype. PI3K-targeting inhibited migration under basal and irradiated conditions through inhibition of HIF-1α, Rho-GTPase expression/activity and localisation whilst having little effect on src/FAK. *In vivo*, radiation induced PI3K, HIF, Rho-GTPases and src but only PI3K, HIF and Rho-GTPases were inhibited by GDC-0941. Co-treatment with GDC-0941 and radiation significantly reduced metastatic dissemination versus radiotherapy alone.

Conclusions: Radiation modifies metastatic characteristics of thyroid carcinoma cells, which can be successfully inhibited by targeting PI3K using GDC-0941 *in vitro* and *in vivo*.

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Radiotherapy is a first line treatment of thyroid carcinoma. The majority of differentiated tumours respond well, however some follicular (FTC) and anaplastic (ATC) thyroid cancers are radioresistant and metastatic. Patients that show recurrent local disease post-radiotherapy usually have lower survival rates due to an increased probability of developing distant metastasis [1–3]. The phosphatidylinositide 3-kinase (PI3K) pathway has been implicated in driving metastatic phenotype in thyroid [4–5] and other cancers [6–8] and PI3K activity is increased by radiotherapy in certain tumours [9–10]. PI3K has pleiotropic effects, forcing metastatic transition via enhanced epithelial-mesenchymal transition (EMT), migration (Vimentin), cell adhesion, spreading (N-cadherin) [8,11] and invasion (matrix-metallo proteinases: MMPs) [12]. Mechanistically the PI3K pathway interacts with the Rho-GTPases (Rac1, Cdc42 and Rho) that regulate the dynamic reorganisation of the actin cytoskeleton [13] and the focal-adhesion associated proteins src and focal-adhesion kinase (src and FAK) [14–16] that promote cell adhesion and movement. We have previously shown that PI3K activates hypoxia-inducible factor-1 (HIF-1) in thyroid tumours and that both pathways contribute significantly to metastatic behaviour in this disease [4,17]. Given the significant link between the PI3K/HIF pathways and metastasis in thyroid carcinoma and the clinical association between post-radiotherapy recurrent and distant metastasis, we assessed the effect of radiotherapy on the metastatic behaviour of FTC/ATC cell lines and FTC tumours with hyperactive PI3K signalling. We further explored how PI3K inhibition via the clinically relevant inhibitor GDC-0941 mechanistically impacted on these phenotypes, with particular attention to src/FAK, the Rho-GTPases and HIF-1 activation.

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Materials and methods

Cell lines

ATC (8505c), FTC (FTC133, WRO) and derivative cell lines (including gifts of FTC133 PTEN and PCI-NEO from Professor Oliver Gimm, University of Linkoping, Sweden) are described previously [4].

Scratch-wound migration assay

Protocols were adapted from those previously described [4]. Briefly, scratched monolayers of quiescent cells were incubated with media supplemented with 0.2% foetal calf serum (FCS) containing either GDC-0941 (Chemdea, NJ, USA) or DMSO (Sigma–Aldrich, Gillingham, UK) for 4 h, irradiated under ambient conditions using a Faxitron X-ray (dose rate 0.952 Gy/min; Faxitron Bioptics, AZ, USA) at 0, 2, 4 or 6 Gray (Gy) and incubated in condition for a further 16 or 20 h depending on study. Images were captured and analysed as previously described [4].

HIF-1 activity assay

An adenoviral-based luciferase reporter assay was used to measure HIF-1 activation as previously described [17]. Briefly infected cells treated with 1 μM GDC-0941 or DMSO were incubated for 18 h, irradiated (0–6 Gy) then incubated for a further 4 h. Cells were lysed and HIF-induced luciferase activity quantified.

Immunofluorescence

Cells were fixed with 10% formalin for 10 min at room temperature (RT), permeabilised with 0.1% Triton X100 in phosphate buffered saline (PBS) for 7 min and blocked with 1% BSA in PBS for 30 min at RT. Cells were incubated with the appropriate primary antibody for 1 h at 37 °C, washed and incubated with a secondary Alexafluor (Life Technologies Ltd., Paisley, UK) for 45 min at 37 °C. Cells were mounted and images obtained at 400× magnification as described above.

Western-blotting

Protocols were used as previously described [17]. For Rho-GTPases/src/FAK analysis 60–70 μg protein was loaded. Protein expression was quantified via densitometry relative to β-actin, using ImageJ.

Primary antibodies

Listed in Supplementary methods.

Xenograft studies

FTC133 cells were implanted sub-cutaneously (0.1 ml of a 5×10^7/ml stock in PBS) in female nude mice (CBA *nu/nu*, aged 8–12 weeks). Radiotherapy commenced when tumours reached 200–250 mm^3 [18]. GDC-0941 was prepared in 0.5% hydroxypropyl methyl cellulose (vehicle) and dosed at 0.1 ml/10 g body weight by oral gavage (p.o.). Mice were dosed twice daily with vehicle or 50 mg/kg GDC-0941 alone or combined with radiation (5 × 2 Gy fractions over 5 consecutive days). Mice received GDC-0941 4 h prior and immediately after radiation. For histology, tumours were rapidly excised 1 h after the final dose of vehicle/GDC-0941 and snap frozen. For metastasis analysis, dosing continued post-radiotherapy for a further 9 days. Once tumours reached maximum burden (approximately 900 mm^3), the lung tissue was excised and analysed for metastatic colonies as described in [4]. All procedures were carried out in accordance with the Scientific Procedures Act 1986 and published guidelines [19] with ethically approved protocols, using the highest standard of welfare under the authority of the Home Office (PPL40/3112, holder Professor K.J. Williams).

Statistical analysis

Student's *t* test and one-way ANOVA with Tukey post-test were used.

Results

Radiation-induced migratory effects are associated with HIF-1 activation and are blocked by PI3K inhibition

We determined if radiation affected the migratory phenotype of follicular (FTC133, WRO) and anaplastic (8505c) thyroid carcinoma cells *in vitro*. Migration increased in a radiation dose-dependent manner in FTC133 and WRO cells (Fig. 1A and Supplementary Fig. 1). Under hypoxia (1% oxygen) consistent with our previous data [4], hypoxia alone induced migration but no further response was observed with combined radiation treatment (Supplementary Fig. 1). In 8505c cells, radiation decreased migratory phenotype in both aerobic and hypoxic conditions (Fig. 1B and Supplementary Fig. 1). These cells exhibit a maximal migratory capacity in air and hypoxia alone has little effect [4]. HIF-1 activity was assessed in response to radiation treatment and correlated with the radiation-induced migratory phenotypes observed (Figs. 1A and B). Inhibition of HIF-1 activity in FTC133s through stable expression of a dominant-negative variant of HIF-1α (dnHIF) abrogated radiation-induced migration (Fig. 1C). Inhibition of PI3K via two independent methods; pharmacological and genetic, abrogated radiation-mediated migration: 1 μM GDC-0941 inhibited both migratory phenotype and the activity of HIF-1 in both FTC133 and 8505c cells (Fig. 1A and B). Re-introduction of PTEN into the PTEN-null FTC133 cells similarly inhibited migration (Fig. 1D).

Inhibition of PI3K via GDC-0941 inhibits HIF-1α and the Rho-GTPases whilst having no effect on the src/FAK axis in vitro

The effects of radiotherapy on activation/expression of PI3K-associated proteins involved in the mechanics of cell migration were ascertained by Western-blot analysis. In both FTC133 and 8505c cells, radiotherapy had little effect on AKT or src activation (pY416), src mediated activation of FAK (pY861), FAK autophosphorylation (pY397), the association of Rac1 with Cdc42 (Rac1/Cdc42-pS7) or expression of Rho (Fig. 2A, Supplementary Fig. 2A). Subcellular localisation of pAKT or pFAK (pY861) was similarly uninfluenced by radiation (Fig. 2B, Supplementary Fig. 2B). However, in both cell types, radiation caused marked changes in the subcellular localisation of Rac1/Cdc42-pS71 and Rho (Fig. 2B and C and Supplementary Fig. 2B and C), with cell lines exhibiting increased accumulation of Rac1/Cdc42-pS71 into focal adhesions in lamellipodia and alterations in Rho subcellular-localisation (Fig. 2B and C, Supplementary Fig. 2B and C). HIF-1α expression was also altered by radiotherapy. To explore how GDC-0941 mechanistically inhibited migration, effects on phosphorylation and activation of the PI3K-associated migratory proteins were assessed. In both cell lines, GDC-0941 had no effect on src/FAK but clearly inhibited the PI3K/HIF-1/Rho-GTPase signalling axis (Fig. 2, Supplementary Fig. 2).

In vivo, radiotherapy increases PI3K, Rho-GTPase, HIF-1 and src activity with GDC-0941 cotreatment inhibiting only the PI3K/HIF-1/Rho-GTPase axis

Mice bearing FTC xenografts were irradiated (2 Gy for 5 consecutive days) with or without additional GDC-0941 treatment (50 mg/kg twice daily) and expression of PI3K-regulated

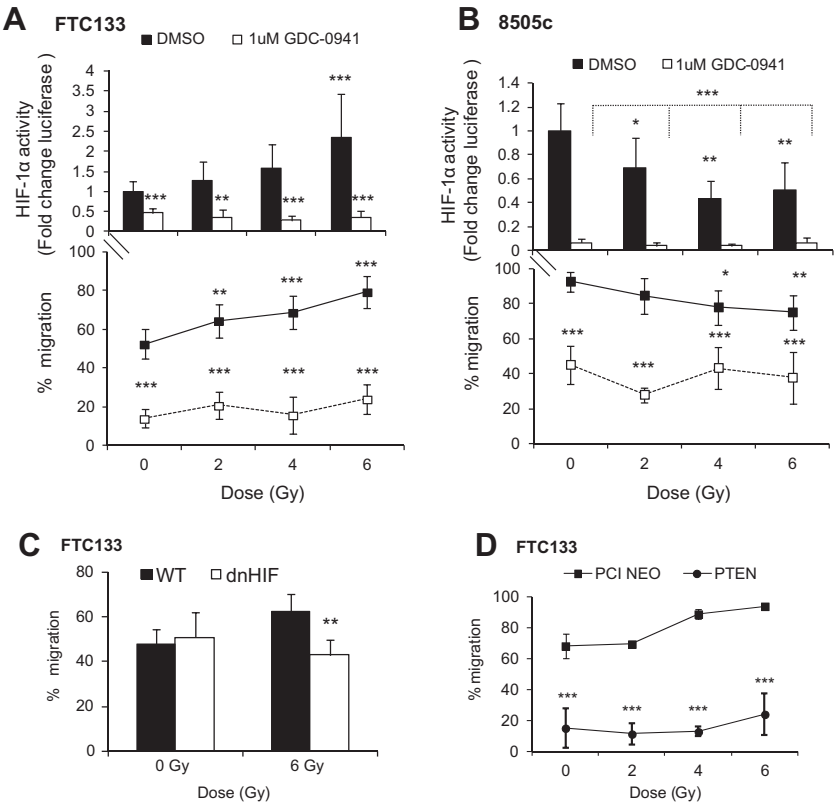

Fig. 1. Radiation modulates migratory potential and HIF activity, which is blocked by PI3K inhibition. (A and B) Radiotherapy dose-dependently increases migration in FTC133 cells and decreases migration in 8505c cells which correlates with changes in HIF-activity. GDC-0941 significantly inhibits migration and HIF-activation. (C) dnHIF expression inhibits radiation-induced migration in FTC133 cells. (D) Genetic inhibition of PI3K via re-introduction of PTEN into PTEN-null FTC133 cells inhibits radiation-induced migration versus vector only (PCI-NEO) control cells. Data represent the mean ± S.D. of 3–4 independent experiments; $^{*}p < 0.01$, $^{**}p < 0.001$, $^{***}p < 0.0001$ versus relevant untreated control.

metastatic proteins assessed by Western-blotting in comparison with size-matched control vehicle treated tumours. In contrast with *in vitro* treatments, radiation clearly induced both PI3K (AKT-pS473) and src (src-pY416; FAK-pY861) in addition to enhancing Rho-GTPase activity/expression. Little evidence of FAK activation was observed (FAK-pY397). Radiation also induced expression of HIF-1α and selective downstream targets associated with metastatic phenotype *in vivo*. GDC-0941 treatment inhibited PI3K, Rho-GTPase activity/expression, HIF-1α and downstream targets Vimentin, MMP-2 and MMP-9 whilst having no effect on src/FAK or N-cadherin (Fig. 3A and B; Supplementary Figs. 3 and 4).

GDC-0941 inhibits metastatic lung colonisation in irradiated FTC xenografts

The FTC133 model spontaneously metastasises to the lung from the primary sub-cutaneous implant site. We previously showed that GDC-0941 inhibited lung metastasis in mice bearing FTC xenografts [4]. To explore the effect of GDC-0941 in combination with radiotherapy on tumour metastasis, tumours were treated as detailed (Methods) and the lung tissue harvested once tumours reached maximum burden (approximately 900 mm^3 achieved on average at days 7, 31 and 35 for control, irradiated and tumours co-treated with GDC-0941 and radiotherapy, respectively). Lung-tumour burden was quantified using an *ex vivo* clonogenic assay [4]. The lungs from mice bearing irradiated tumours had significantly more metastatic colonies compared to non-irradiated controls, which perhaps reflects the relative excision timeframe. When combined with radiation, GDC-0941 halved the metastatic colonisation to the lungs observed with radiation alone (Fig. 3C).

Discussion

Radiotherapy is a first line treatment in many cancers including thyroid but may promote metastasis. Clinically, patients with recurrent local disease post radiotherapy usually have lower survival rates due to increased distant metastasis. Here we provide important evidence that radiotherapy modulates the metastatic phenotype in thyroid carcinoma, which appears dependent on interplay between the PI3K and HIF-1 signalling pathways. PI3K activates HIF-1 in thyroid carcinoma cells, which increases metastatic potential [4]. Here we show that radiation-induced changes in cell motility link to radiation-modulated HIF-1 activity that is dependent on PI3K. Radiation dose-dependently increased HIF-1 activity in FTC133 cells and inhibition of HIF-1 via a dominant negative variant abrogated radiation-induced migratory phenotype. In 8505c cells where radiotherapy reduced HIF-1 activity, also reduced migratory phenotype, further supporting that HIF activity relates directly to migratory phenotype. It is important to note that 8505c's are primed for maximal migration under normoxia and basal HIF-1 activation in this cell line is higher compared with FTC133s cells [17]. This may reflect the relative differences observed between the two cell lines in HIF-mediated responses to radiotherapy. The use of GDC-0941, a clinically relevant inhibitor of PI3K inhibited both HIF-1 activity and migratory phenotype in follicular and anaplastic thyroid cell lines. Mechanistically we show that the anti-migratory effects of GDC-0941 appear elicited

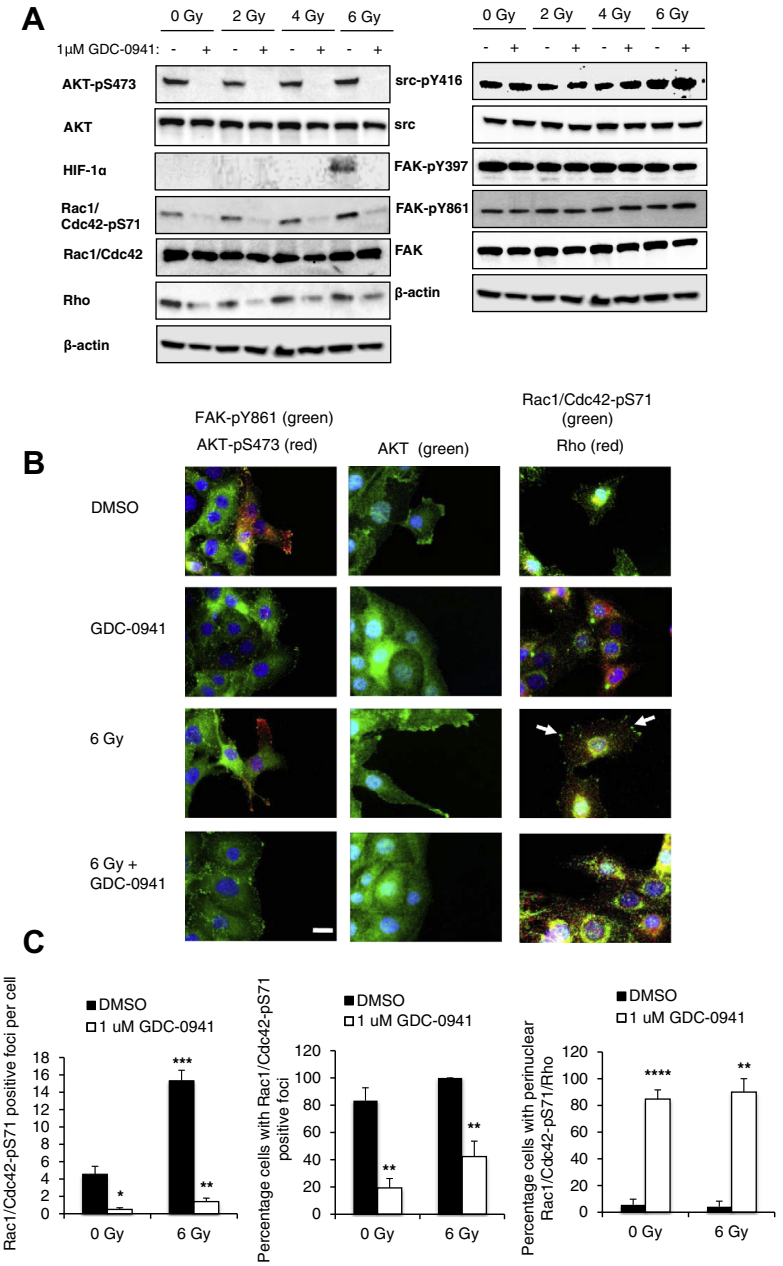

Fig. 2. *In vitro*, radiotherapy has little effect on expression or activation of PI3K, src/FAK or Rho-GTPases, but causes significant changes in subcellular localisation of Rho-GTPases and expression of HIF-1α PI3K inhibition via GDC-0941 inhibits PI3K, HIF-1α and Rho-GTPases activity/expression whilst having no effect on src/FAK. (A) Western blot data shown for FTC133 cells treated for 18 h with DMSO (−) or 1 μM GDC-0941 (+) which were then irradiated (0, 2, 4, 6 Gy) and incubated for a further 4 h in condition. β-actin was used as the loading control. (B) Confluent cell monolayers were scratched to generate a 'wound' and incubated with 1 μM GDC-0941/DMSO for 4 h, irradiated (6 Gy) and left for 16 h. Arrows indicate Rac-1/Cdc42-pS71 accumulation at focal adhesion points induced by radiotherapy. (C) Quantification of Rac-1/Cdc42-pS71 foci and subcellular localisation of Rac-1/Cdc42-pS71 expression in cells treated as in (B). Radiotherapy significantly increases Rac-1/Cdc42-pS71 positive foci ($^{*}p < 0.00001$). GDC-0941 significantly inhibits foci number and percentage cells with Rac-1/Cdc42-pS71 positive foci and significantly increases perinuclear localisation of Rac-1/Cdc42-pS71 in both non-irradiated and irradiated cells ($^{*}p < 0.001$, $^{**}p < 0.0001$, $^{***}p < 0.000001$, $^{****}p < 0.00000001$). Nuclei were stained with DAPI (blue). Scale bar: 20 μm. Blots and images are representative of three independent experiments.

via impact on the Rho-GTPase axis whilst having little effect on src or FAK. These data are supported by work showing that inhibition of the PI3K-regulated protein mammalian target of rapamycin (mTOR) by rapamycin also inhibited expression of the Rho-GTPases [20]. Conversely to GDC-0941, rapamycin also inhibited FAK activation. Our data suggest that GDC-0941 blocks migration via selective targeting of two distinct signalling axes: PI3K/Rho-GTPase and PI3K/HIF-1.

Radiation treatment *in vitro* did not alter expression/activity of PI3K, src/FAK or Rho-GTPases but did cause marked changes in the subcellular localisation of the latter proteins. Localisation of the Rho- GTPases differed between the FTC133 and 8505c cells, which, in addition to differences in HIF-1, may contribute to the observed differences in migratory phenotype. In contrast, radiation treatment of FTC133 xenografts *in vivo* caused activation of PI3K, src and Rho-GTPases as well as inducing HIF-1α and downstream targets. FAK autophosphorylation was not observed, suggesting that FAK is not fully activated by radiotherapy in this model. The striking differences between pathway activation *in vitro* and *in vivo* likely reflect the contribution of the extracellular matrix in the

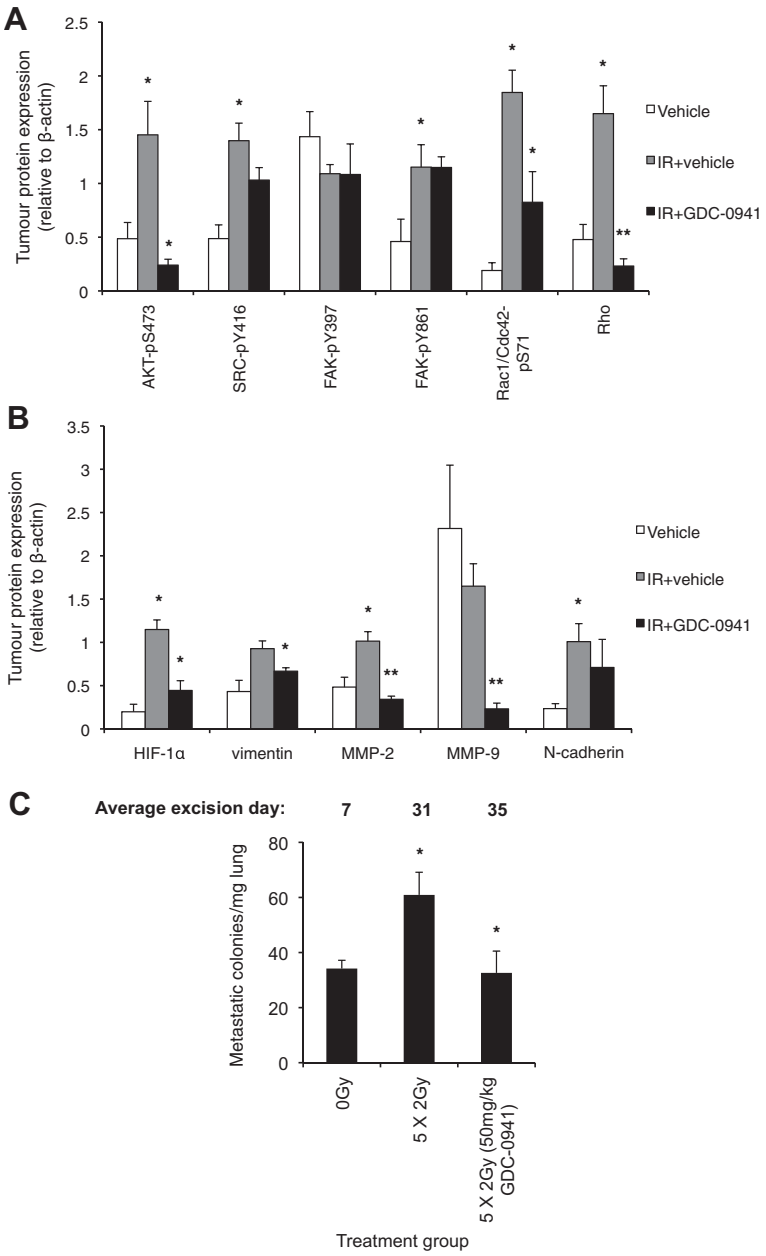

Fig. 3. GDC-0941 inhibits radiation-induced phosphorylation of AKT, Rac1/Cdc42 and Rho expression, but has little effect on src/FAK in FTC133 xenografts and reduces lung metastatic dissemination. Densitometry analysis of the expression of PI3K/metastases associated proteins (A) and HIF/HIF-downstream metastatic-targets (B) relative to β-actin controls in control, irradiated (5×2 Gy) and GDC-0941-treated irradiated tumours. Data represent the mean ± S.E.M following analysis of the blots given in supplementary Figs. 3 and 4. $^{*}p < 0.05$, $^{**}p < 0.01$; radiotherapy (IR) samples were compared to control and GDC-0941+ radiotherapy to radiotherapy only. (C) Analysis of tumour clonogens isolated from the lungs of control vehicle-treated mice (0 Gy; $n = 4$) and mice treated with 5×2 Gy radiotherapy alone ($n = 4$) or with GDC-0941 ($n = 5$) that were isolated when primary tumour volumes exceeded 900 mm^3 (numbers above the bars indicate when this was achieved for the different treatment groups). Data represent the mean ± S.E.M.

latter case, which is well known to influence cell survival following radiation treatment [21]. GDC-0941 inhibited radiation-induced PI3K, HIF-1 and Rho-GTPase activity, but, as in the *in vitro* scenario, had no effect on src. Although previous reports have suggested PI3K-mediated activation of src [14]: this does not occur following radiation treatment of follicular thyroid carcinoma xenografts. Consistent with the inhibitory effects of GDC-0941 on migratory phenotype *in vitro*, GDC-0941 reduced metastatic dissemination of FTC133 xenografts *in vivo*. Radiation alone saw an increase in lung tumour clonogens compared with control tumours. However this cannot be interpreted simply as radiation promoting metastatic phenotype as the radiation treatment induces a substantial delay in tumour growth such that the lung burden can accumulate over an increased period of time. Lung dissemination was halved with GDC-0941 and there was no detrimental effect to the primary tumour response with the co-treatment. Indeed in these animals, tumour excision was delayed by 4 days compared with radiotherapy alone. These data support the development of PI3K inhibition in combination with radiotherapy to improve overall disease free survival.

Conflict of interest statement

No conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.radonc.2013.06.027.

References

[1] Busaidy NL, Cabanillas ME. Differentiated thyroid cancer: management of patients with radioiodine nonresponsive disease. J Thyroid Res 2012;2012:618985.
[2] Jones MK. Management of papillary and follicular thyroid cancer. J R Soc Med 2002;95:325–6.
[3] Vorster M, Sathekge MM. Detection of extensive metastases from anaplastic thyroid cancer by F-18 FDG-PET/CT. Open Nucl Med J 2011;3:1–6.
[4] Burrows N, Babur M, Resch J, Ridsdale S, Mejin M, Rowling EJ, et al. GDC-0941 inhibits metastatic characteristics of thyroid carcinomas by targeting both the phosphoinositide-3 kinase (PI3K) and hypoxiainducible factor-1alpha (HIF-1alpha) pathways. J Clin Endocrinol Metab 2011;96:E1934–43.
[5] Paes JE, Ringel MD. Dysregulation of the phosphatidylinositol 3-kinase pathway in thyroid neoplasia. Endocrinol Metab Clin North Am 2008;37:375–87 [viii–ix].
[6] Jiang BH, Liu LZ. PI3K/PTEN signaling in angiogenesis and tumorigenesis. Adv Cancer Res 2009;102:19–65.
[7] Qiao M, Iglehart JD, Pardee AB. Metastatic potential of 21T human breast cancer cells depends on Akt/protein kinase B activation. Cancer Res 2007;67:5293–9.
[8] Larue L, Bellacosa A. Epithelial-mesenchymal transition in development and cancer: role of phosphatidylinositol 3′ kinase/AKT pathways. Oncogene 2005;24:7443–54.
[9] Wild-Bode C, Weller M, Rimner A, Dichgans J, Wick W. Sublethal irradiation promotes migration and invasiveness of glioma cells: implications for radiotherapy of human glioblastoma. Cancer Res 2001;61:2744–50.
[10] Pickhard AC, Margraf J, Knopf A, Stark T, Piontek G, Beck C, et al. Inhibition of radiation induced migration of human head and neck squamous cell carcinoma cells by blocking of EGF receptor pathways. BMC Cancer 2011;11:388.
[11] Jo M, Lester RD, Montel V, Eastman B, Takimoto S, Gonias SL. Reversibility of epithelialmesenchymal transition (EMT) induced in breast cancer cells by activation of urokinase receptor-dependent cell signaling. J Biol Chem 2009;284:22825–33.
[12] Park BK, Zeng X, Glazer RI. Akt1 induces extracellular matrix invasion and matrix metalloproteinase-2 activity in mouse mammary epithelial cells. Cancer Res 2001;61:7647–53.
[13] Cain RJ, Ridley AJ. Phosphoinositide 3-kinases in cell migration. Biol Cell 2009;101:13–29.
[14] Walker VG, Ammer A, Cao Z, Clump AC, Jiang BH, Kelley LC, et al. PI3K activation is required for PMA-directed activation of cSrc by AFAP-110. Am J Physiol Cell Physiol 2007;293:C119–32.
[15] Reiske HR, Kao SC, Cary LA, Guan JL, Lai JF, Chen HC. Requirement of phosphatidylinositol 3-kinase in focal adhesion kinase-promoted cell migration. J Biol Chem 1999;274:12361–6.
[16] Campbell M, Trimble ER. Modification of PI3K- and MAPK-dependent chemotaxis in aortic vascular smooth muscle cells by protein kinase CbetaII. Circ Res 2005;96:197–206.
[17] Burrows N, Resch J, Cowen RL, von Wasielewski R, Hoang-Vu C, West CM, et al. Expression of hypoxia-inducible factor 1 alpha in thyroid carcinomas. Endocr Relat Cancer 2010;17:61–72.
[18] Williams KJ, Telfer BA, Shannon AM, Babur M, Stratford IJ, Wedge SR. Combining radiotherapy with AZD2171, a potent inhibitor of vascular endothelial growth factor signaling: pathophysiologic effects and therapeutic benefit. Mol Cancer Ther 2007;6:599–606.
[19] Workman P, Aboagye EO, Balkwill F, Balmain A, Bruder G, Chaplin DJ, et al. Guidelines for the welfare and use of animals in cancer research. Br J Cancer 2010;102:1555–77.
[20] Liu L, Luo Y, Chen L, Shen T, Xu B, Chen W, et al. Rapamycin inhibits cytoskeleton reorganization and cell motility by suppressing RhoA expression and activity. J Biol Chem 2010;285:38362–73.
[21] Eke I, Cordes N. Radiobiology goes 3D: how ECM and cell morphology impact on cell survival after irradiation. Radiother Oncol 2011;99:271–8.