Receptors & Clinical Investigation 2015; 2: e664. doi: 10.14800/rci.664; © 2015 by Pia Giovannelli, *et al*.
http://www.smartscitech.com/index.php/rci

RESEARCH HIGHLIGHT

The dual role of androgen receptor in mesenchymal cells

Pia Giovannelli, Marzia Di Donato, Gustavo Cernera, Annalisa Di Santi, Giovanni Galasso, Erika Di Zazzo, Flavia Vitale, Gabriella Castoria, Antimo Migliaccio

Department of Biochemistry, Biophysics and General Pathology, II University of Naples, Via L. De Crecchio, Naples 780138，Italy

Correspondence: Gabriella Castoria
E-mail: gabriella.castoria@unina2.it
Received: February 23, 2015
Published online: April 11, 2015

The androgen receptor (AR) mediates differentiation, proliferation and transformation of target tissues. These processes require a crosstalk between epithelial and stromal cells. Prostate cancer (PCa) represents a major cause of cancer-related mortality in men, and is often associated with deregulation of androgen/AR axis. Clinical and molecular findings have highlighted the role of epithelial AR in PCa progression. In contrast, the functions of AR in mesenchymal cells are still unclear. We previously reported that low androgen concentration (1 pM) triggers interaction of AR with the Src tyrosine kinase and PI3-K, thus driving cell cycle progression in fibroblasts. In contrast, stimulation of fibroblasts and fibrosarcoma cells with physiological (10 nM) androgen concentration leads to interaction of AR with full-length filamin A (FLNa) and does not trigger DNA synthesis. On the basis of these findings, we re-examined the role of androgen/AR axis in fibroblasts and human fibrosarcoma HT1080 cells. Recently, we obtained two original and integrated findings on the decision of mesenchymal cells to undergo reversible quiescence and migrate upon stimulation with 10 nM androgens (Castoria *et al*. 2011 and 2014). This decision is dependent upon the interaction of AR with FLNa. Once assembled, the bipartite AR/FLNa complex recruits β1-integrin and triggers Rac1 activation, thereby enhancing on the one hand cell motility. On the other, Rac 1 activation triggers its downstream effector DYRK 1B, which phosphorylates Ser10 of p27. Stabilization of p27 and cell quiescence then follow. These results strengthen and extend our studies, adding a new and exciting piece to the complex puzzle of signaling networks activated by androgens in target cells. Our findings might have implications for current approaches to AR-related diseases.

Keywords: androgen receptor; Src; PI3-K; filamin A; Rac 1; DYRK 1B; cell motility; cell cycle

To cite this article: Pia Giovannelli, *et al*. The dual role of androgen receptor in mesenchymal cells. Receptor Clin Invest 2015; 2: e664. doi: 10.14800/rci.664.

Migration/proliferation dichotomy in androgen action

Cell proliferation and motility are mutually exclusive processes. This mechanism, also known as the 'go or grow' process, is conserved across very different species. It is involved, for instance, at the edge of species extinction, when high motility might represent an advantage compared with the limit of low density [1]. Again, some insects [2] and fish [3] exhibit the same behavior under restricted conditions of food availability. It is largely accepted that moving cells have a low proliferation rates. Conversely, cells usually proliferate when they do not move. Depending on the spatial and dynamic organization of signaling networks, ligand concentration, expression and localization of cognate receptors, growth factors (i.e, EGF, VEGF) influence whether cells migrate, differentiate or divide [4, 5, 6, 7]. More importantly, this mechanism is involved in human cancer progression, when highly proliferating cells acquire an invasive phenotype [8].

We previously observed that a low (1 pM) concentration of androgens, R1881 or DHT, triggers the S-phase entry,

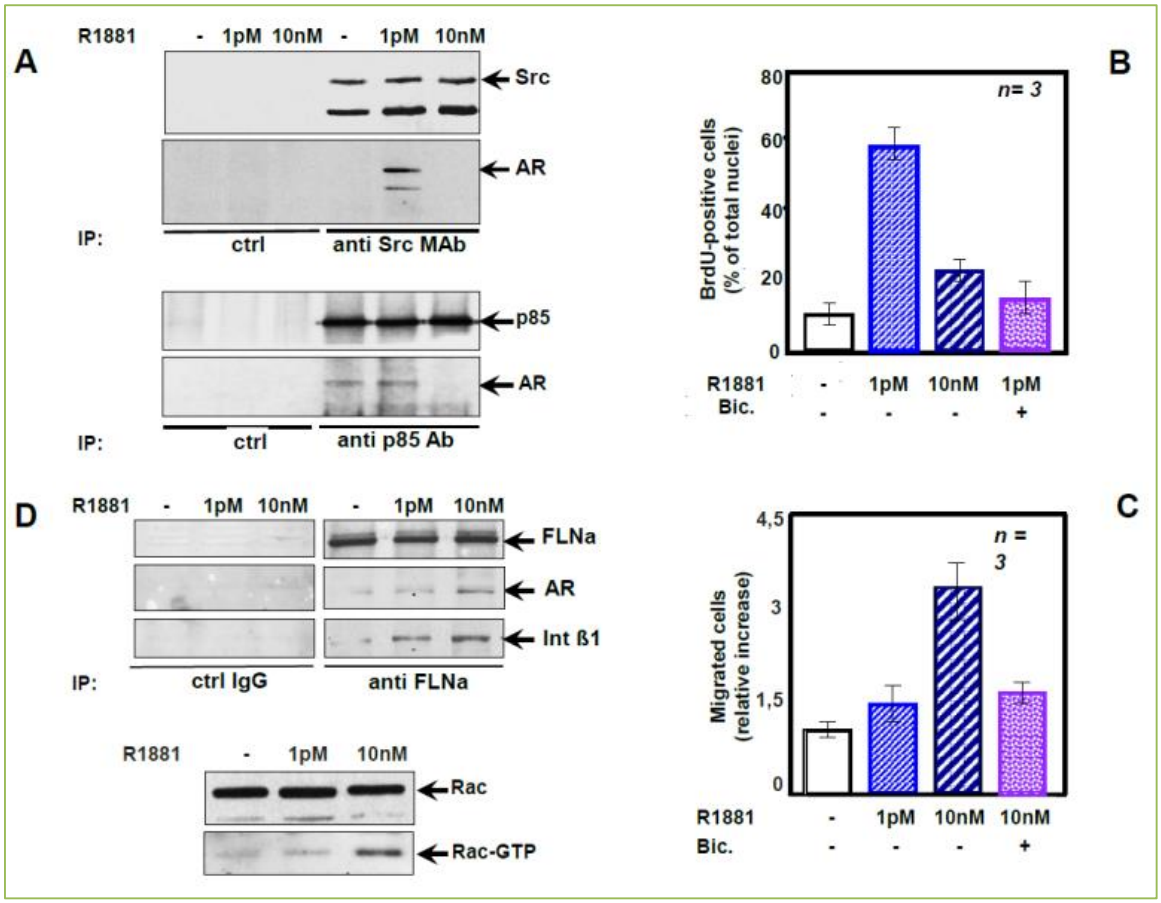

Figure 1. The dichotomous effect of androgens in NIH3T3 fibroblasts. Quiescent NIH3T3 fibroblasts were used. In A and D, cells were left unstimulated or stimulated for 5 min with the indicated R1881 concentrations. Lysate proteins were immunoprecipitated with the anti Src (upper panel A) or anti p85 (lower panel in A) or anti FLNa (D) antibodies. Control lysate proteins were immunoprecipitated with non-specific antibodies (ctrl). Proteins in immune complexes were resolved by SDS-PAGE, immunoblotted and then probed with the antibodies against the indicated proteins. Using an NIH 1.61 image program, a 38% increase in AR/p85 association was detected on 1 pM R1881 stimulation of cells. This experiment was reproduced with similar findings. Lysate proteins were also used to detect Rac1 activation, using pull-down assay (lower panel in D). Quiescent NIH3T3 cells were left untreated or treated for 18 h (B) or 6 h (C) with the indicated R1881 concentrations, in the absence or presence of 10 microM bicalutamide. In B, the cells were pulsed with BrdU (100 □icroM). BrdU incorporation was analyzed by immunofluorescence and expressed as % of total nuclei. In C, the cells were allowed to migrate in Transwell chambers. Migrated cells were stained with Hoechst, counted by fluorescent microscope, and expressed as relative increase.

whereas physiological (10 nM) androgen concentration activates migration and does not drive DNA synthesis in NIH3T3 fibroblasts [9]. A similar dichotomous response (migration/proliferation dichotomy) was subsequently described in the same cells challenged with low or high PDGF concentration, and was attributed to the different types of endocytosis (clathrin- or non-clathrin-mediated) and degradation engaged by PDGF receptor [10].

Our findings indicated that fibroblasts switch from a proliferative to a migratory phenotype when androgen concentration reaches a critical threshold. Although exciting, these results raised a number of questions. Firstly, we asked when and how androgen levels undergo fluctuations. We reflected on the various examples of androgen level changes in blood, including circadian rhythm of testosterone, age-related androgen deficiency, low androgen concentration in hypogonadism, and acute reduction of androgen levels following chemical or surgical castration. Although in most of these examples fluctuations in androgen levels are mild, a more pronounced hormone concentration change might induce a dramatic shift, or even paradoxical effects in the resulting biological responses. A clinical example is the acceleration of metastatic spreading in PCa patients who early receive the anti-androgen bicalutamide, as an androgen-depleting treatment [11]. Again, the biphasic androgen effect we observed might play a role during male sexual differentiation when presumptive peritubular myoid cells (PTMCs) reduce their proliferation rate and begin to migrate from mesonephros into the testis [12]. In adult life,

such a switch might occur during PCa metastasis, when local androgen levels increase [13], or during inflammation and wound healing. A similar dichotomy, however, has been reported in highly metastatic pancreas cancer-derived cells, which are almost insensitive to androgens in terms of cell proliferation, while they efficiently migrate in response to hormone treatment [14]. We also observed a similar shift in PCa-derived epithelial cells, when they progress towards a hormone-independent state *(unpublished results*). However, other tumor cell types (i.e., colon and lung cancers) only slightly respond to androgens with proliferation. Under certain conditions, these hormones even block cell transformation [15].

In conclusion, our findings highlight the conservation of this process across quite different cell types and also point to a different role for androgens during the epithelial-mesenchymal transition (EMT) of cancer cells. It can be speculated that androgens prevalently promote cell proliferation in cells with the epithelial phenotype, whereas they stimulate motility after the cells undergo EMT.

Migration/proliferation dichotomy in androgen action: the role of androgen-activated AR/Src/PI3-K complex

The different biological outcomes elicited by low and high androgen levels led us to dissect the pathways activated by the two hormone concentrations. We firstly hypothesized that the observed dichotomy is due to transcriptional or non-transcriptional effects triggered by androgens in fibroblasts. However, by analyzing both ligand-induced AR nuclear translocation and activation of gene transcription in NIH3T3 fibroblasts [9], we surprisingly discovered that AR expressed in these cells does not enter nuclei and is devoid of transcriptional activity. Similar findings were then observed in MEFs as well as fibrosarcoma cells [15, 16]. Thus, in mesenchymal and transformed mesenchymal cells AR appeared as a 'non-functional' receptor. Other groups have reported similar results. AR from prostate stromal cells weakly mediates gene transcription and proliferation in response to physiological DHT concentration [17, 18]. Again, it has been recently shown that AR expressed in prostate stromal WPMY-1 cells is permanently localized in cytoplasm and is devoid of transcriptional activity [19]. Notably, WPMY-1 cells have been used in combination with PCa epithelial PC-3 cells in a mouse model of prostate tumorigenesis. These studies concluded that stromal but not epithelial AR promotes tumor proliferation at very early stage [11]. Thus, AR expressed in stromal WPMY-1 cells fosters tumor growth, likely through a non-transcriptional mechanism. These results further point to the role of non-transcriptional action mediated by AR in stromal cells.

By investigating the mechanism responsible for cell proliferation observed in NIH3T3 cells, we discovered that 1 pM R1881 induces association of AR with Src (Fig.1, upper panel in A). At this hormone concentration, a weaker but still significant increase in association of AR with p85, the regulatory subunit of PI3-K, was also observed (Fig.1, lower panel in A and Figure legend). We then showed that the androgen-induced ternary AR/Src/p85 complex activates downstream Erk-2 and Akt effectors. In turn, Erk-2 induces p27 cytoplasmic release [9, 20], while Akt increases cyclin D1 transcription [9, 21]. In such a way, the cells enter S-phase (Fig.1B). The anti-androgen bicalutamide inhibits BrdU incorporation induced by 1 pM R1881 in NIH3T3 cells (Fig.1B). We observed similar data in primary fibroblasts from embryo or adult mouse, indicating that our findings are not restricted to immortalized cells [9, 15, 16].

The AR/Src/PI3-K complex assembly is consistent with our previous findings in hormone dependent epithelial cells, as the AR/Src complex was originally observed in PCa-derived LNCaP cells challenged with androgens [22]. Again, others and we observed the assembly of a ternary complex made up of estradiol receptor alpha (ERα), Src and PI3-K in estradiol-stimulated breast cancer-derived MCF-7 cells [21, 23]. In both cell types, association of AR or ERα with Src and p85 induces activation of a downstream pathway driving G1-S progression of cell cycle [20, 21, 22, 23]. Other groups confirmed these findings in several breast cancer cells [24, 25], as well as in other tissues [26]. It was later shown that overexpression of the ERα/Src/PI3-K complex correlates with malignancy and aggressiveness in a subset of human breast cancers [27].

After many years of investigation, it is now recognized that interaction of steroid receptors (ERα or β, AR and PR) with Src and PI3-K has a key role in sex steroid rapid action [28, 29, 30]. These findings might lead to identification of new targets in hormone-dependent cancers. By specifically targeting interaction of AR or ERα with Src, small peptides inhibiting breast and prostate tumor growth [31, 32] could be used advantageously alone or in combination with classic endocrine therapy in clinical trials.

Migration/proliferation dichotomy: the role of androgen-activated AR/FLNa complex

The discovery that the migration/proliferation dichotomy depends on androgen concentration led us to investigate the mechanism responsible for the shift towards a migratory phenotype in fibroblasts. We found that fibroblasts challenged with 10 nM R1881 do not enter S-phase (Fig.1B) and undergo a reversible state of quiescence [15]. At the same time, we observed cytoskeleton changes [9, 16] and

enhancement of cell migration (Fig.1C) [16]. Notably, at this hormone concentration we detected dissociation of AR from Src and PI3-K (upper and lower panels in Fig.1A), thus indicating that AR/Src/PI3-K complex assembly is not involved in the cell's decision to halt cell cycle and migrate.

In an attempt to provide a mechanistic insight into this shift, we hypothesized the involvement of FLNa. By interacting with a variety of proteins (i.e., integrins, Trio-GEF, Fil-GAP, Rac), this scaffold protein commands cell motility [33]. Moreover, FLNa and its proteolytic fragments directly interact with AR to regulate its nuclear translocation [34] or transcriptional activity [34, 35], or even the androgen-dependence of PCa LNCaP cells [36]. The co-immunoprecipitation experiment presented in Fig.1D (upper panel) shows that stimulation of NIH3T3 cells with 10 nM R1881 significantly increases the association of AR with both full-length FLNa and β1-integrin. This complex strongly enhances Rac activity (Fig.1D, lower panel). Notably, 1 pM R1881 only slightly triggers cell motility (Fig.1C), does not significantly increase AR association with FLNa, and weakly stimulates AR association with β1-integrin (upper panel in Fig.1D). Interestingly, this hormone concentration does not activate Rac (lower panel in Fig.1D). Thus, the shift in AR interaction (Src/PI3-K *versus* FLNa) accounts for the observed change in cell outcome (proliferation *versus* migration). In sum, data presented in Fig.1, together with additional analyses above reported [15, 16], indicate that 10 nM R1881 induces assembly of the AR/FLNa bipartite complex, which then recruits β1-integrin. On one hand, the 10 nM R1881-induced AR/FLNa/β1-integrin complex recruits and activates Rac. On the other, the same complex activates the focal adhesion kinase (FAK) and its downstream pathway [16]. Noteworthy, activation of Rac 1 and FAK are mutually exclusive, since the silencing or the chemical inhibition of either FAK or Rac1 induces a stronger activation of the other effector. Such a control ensures that the upstream AR/FLNa/β1-integrin complex mutually regulates activation of FAK or Rac, enabling a dynamic regulation between focal complex turnover and cytoskeleton changes. By this way, the migration speed of cells is finely modulated [16].

In conclusion, we reported for the first time that AR links androgen signaling to FLNa and β1-integrin in mesenchymal cells challenged with physiological hormone concentration. This FLNa-mediated signaling likely overcomes the hormone proliferative effects observed at picomolar concentrations of androgen. Furthermore, we described an 'inside-out' mechanism of integrin activation by a sex steroid hormone, with implications in migration, development and metastatic processes. Our preliminary data suggest that assembly of the AR/FLNa/β1-integrin complex is involved in neuronal differentiation induced by androgens (*submitted*) and metastatic spreading of different androgen-responsive cancer cell types (*unpublished*).

Our findings, however, call for additional comments. Primordial germ cells from null-β1-integrin mouse do not migrate to the gonads [37], mimicking the phenotype of male AR-knockout mice that exhibits severe developmental defects [38]. Thus, the β1-integrin functions might intersect androgen/AR axis during development and metastatic processes. FLNa is an important piece in this puzzle. A calpain-generated FLNa fragment was identified as an AR co-regulator in cell nuclei [34, 35, 36]. However, in contrast with data obtained in PCa-derived epithelial cells [36, 39, 40], we were unable to detect nuclear localization [16] and proteolysis (*unpublished*) of FLNa in NIH3T3 fibroblasts. In fact, stimulation with physiological androgen concentration increases by about 3-fold the co-localization ratio between AR/FLNa at intermediate cytoskeleton filaments of NIH3T3 fibroblasts [15, 16]. These findings suggest that intracellular localization of the AR/FLNa complex commands the response of cells triggered by androgens, since it controls motility when localized in cytoplasm, while it modulates the gene transcription in nuclei. The finding that high levels of cytoplasmic FLNa can be detected in metastatic PCa [39] indicates that cytoplasmic localization of the AR/FLNa complex is re-capitulated during progression and metastatic spreading of PCa. The behavior of highly metastatic human fibrosarcoma HT1080 cells, which undergo a robust migration when challenged with androgens [15, 16, 41], favors this hypothesis.

Altogether, these findings suggest that the cytoplasmic AR/FLNa complex potentially represents a novel biomarker to predict aggressiveness of PCa or even its response to endocrine therapies. Notably, a stapled peptide, which displaces the AR/FLNa complex and inhibits Rac activation impairs the migratory functions induced by physiological androgen concentration in target cells (Fig.2A) [15]. These encouraging results warrant further investigation based on the use of this peptide in preclinical and clinical studies.

Ten nM androgens activate DYRK 1B and induce reversible quiescence in fibroblasts

Dual-specificity tyrosine-phosphorylation-regulated kinases (DYRKs) are involved in many cellular processes [42]. These kinases control cell cycle and differentiation through phosphorylation of cell cycle regulators [42]. A member of the DYRK family, DYRK 1B induces reversible quiescence by stabilizing p27 through Ser10 phosphorylation. In addition, small GTP-binding proteins of the Rho family control DYRK 1B [43, 44].

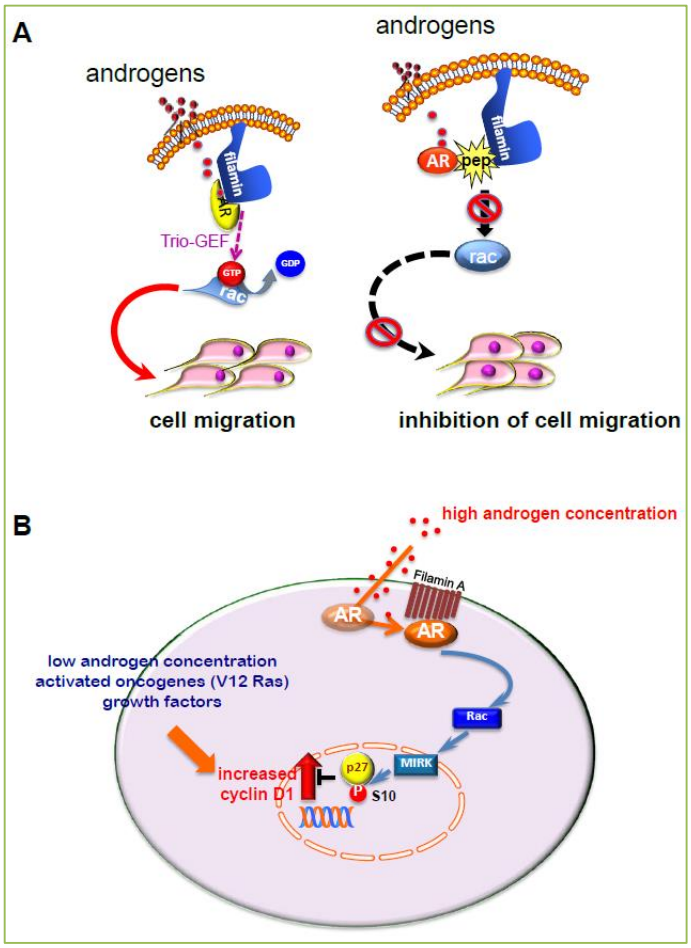

Figure 2. Molecular mechanism responsible for migration/ proliferation dichotomy induced by different androgen concentration in NIH3T3 cells. In A, stimulation of NIH3T3 cells with 10 nM androgens induces the assembly of AR/FLNa complex in the extra-nuclear compartment of cells. This complex likely recruits Trio-GEF to activate Rac and induce cell migration (left panel) [16]. A stapled peptide, mimicking the AR sequence responsible for AR interaction with FLNa, disrupts the assembly of AR/FLNa complex, thereby inhibiting Rac1 activation and cell motility in 10 nM androgen-treated NIH3T3 cells (right panel) [16]. In B, low androgen concentration (1 pM) triggers cyclin D1 transcription and cell proliferation through the AR/Src/p85□lpha complex assembly in NIH3T3 cells [9]. High androgen concentration (10 nM) activates the FLNa/Rac/DYRK 1B cascade. DYRK 1B activation results in p27 Ser10 phosphorylation and p27 stabilization. Quiescence of fibroblasts then follows [15]. Thus, high androgen concentration (10 nM) offsets the growth-promoting action of low androgen concentration, or transformation induced by oncogenic Ras in mesenchymal cells [15]. Mitogenic signaling elicited by growth factors is also inhibited (unpublished).

In 10 nM androgen-treated NIH3T3 cells, we observed congruent effects on Rac and DYRK 1B activation as well as p27 Ser10 phosphorylation and DNA synthesis. Ten nM R1881, but not 1 pM R1881 activates Rac1 (lower panel in Fig.1D), increases activity of DYRK 1B and p27 Ser10 phosphorylation [15], but does not significantly affect BrdU incorporation in NIH3T3 cells (Fig.1B). In contrast, 1 pM R1881 does not affect DYRK 1B activity and does not trigger p27 Ser10 phosphorylation [15]. At that hormonal concentration, indeed, DNA synthesis is robustly increased (Fig.1B). Thus, activation of DYRK 1B in fibroblasts challenged with 10 nM R1881 would result in p27-Ser10 phosphorylation, followed by p27 stabilization and quiescence. Somatic knock-down of DYRK 1B significantly increases BrdU incorporation and almost completely blocks p27 Ser10 phosphorylation in 10 nM R1881-treated NIH3T3 cells [15], supporting the role of DYRK 1B in p27 Ser10 phosphorylation. Findings recently collected in our lab have shown show that Rac1-dependent activation of DYRK 1B triggers p27 Ser10 phosphorylation, thereby inducing its stabilization and G0 arrest in 10 nM R1881-treated cells [15]. Since the upstream AR/FLNa complex controls both Rac1 and DYRK 1B activation, the FLNa/Rac1/DYRK 1B pathway triggered by 10 nM androgen halts cell cycle through p27 Ser10 phosphorylation [15]. Stimulation of mesenchymal cell proliferation by 10 nM androgen is, indeed, observed upon AR/ FLNa/Rac1/DYRK 1B pathway inhibition, indicating that high (10 nM) androgen levels overcomes the proliferative circuitry activated by low (1 pM) androgen concentration in fibroblasts (Fig.2B). Thus, 10 nM androgens activate the FLNa/Rac1/DYRK 1B cascade to offset their growth, promoting action in mesenchymal cells, or inhibit mitogenic signaling elicited by growth factors (i.e., EGF; *unpublished*) or transformation induced by oncogenic Ras [15]. In sum, AR could inhibit cell growth by interfering in growth factor- or Ras-driven pathways (Fig.2B), without affecting cell proliferation triggered by different signaling mechanisms (i.e., serum or oncogenic Src) [15].

DYRK 1B is activated by oncogenic Ras through the Rac11/MKK3 signaling pathway, and DYRK 1B activation has been reported in pancreatic, ovarian and colon cancers. Thus, DYRK 1B emerges as an important player in transformation induced by oncogenic Ras and tumor progression [45]. DYRK 1B re-directs the autocrine toward paracrine hedgehog signaling in a mouse model of pancreatic cancer [46]. By enabling the growth of stromal cells through hedgehog signaling, DYRK 1B inhibition impairs the growth of pancreatic cancer in a mouse model [44]. The discovery that androgen/AR axis controls DYRK 1B activation in fibroblasts is significant, since AR/DYRK 1B pathway inhibition might enhance fibroblast growth and collagen content, thereby limiting tumor growth *in vivo*.

In conclusion, these findings strongly encourage further dissection of androgen/AR axis in the stromal compartment of PCa *in vivo*. Inhibition of AR functions by small peptides that specifically disrupt the AR/FLNa interaction [15] or new AR pan-antagonists [47] might open up new horizons to specifically modulate the functions of stromal AR and restrain the growth of AR-expressing cancers.

Acknowledgements

The Italian Association for Cancer Research (IG11520 to A.M.), and the Italian Ministry of University and Scientific Research (P.R.I.N. 2010-2011; 2010NFEB9L_002 to G.C.) supported this work. Pia Giovannelli, Marzia Di Donato and Erika Di Zazzo are supported by a fellowship of the Italian Ministry of University and Scientific Research (P.R.I.N. 2010-2011).

Author contributions

G.C. and A.M. contributed to concept and design of the study and writing of the review; P.G., M.D.D., G.C., A.D.S., G.G., E.D.Z. and F.V. performed all the experiments presented and contributed to analysis of data.

Conflict of interests

The authors declare that they do not have any competing financial interests in relation to the work described.

References

1. Viswanathan GM, Raposo EP, da Luz MGE. Levy flights and superdiffusion in the context of biological encounters and random searches. Phys Life Rev 2008; 5:133-150.
2. Sisterson MS, Averill AL. Costs and benefits of food foraging for a braconid parasitoid. J Insect Behav 2002; 15:571-588.
3. Lamine K, Lambin M, Alauzet C. Effect of starvation on the searching path of the predatory bug deraeocoris lutescens. BioControl 2005; 50:717-727.
4. Chen P, Gupta K, Wells A. Cell movement elicited by epidermal growth factor receptor requires kinase and autophosphorylation but is separable from mitogenesis. J Cell Biol 1994; 124:547-555.
5. Chen P, Xie H, Sekar MC, Gupta K, Wells A. Epidermal growth factor receptor-mediated cell motility: phospholipase C activity is required, but mitogen-activated protein kinase activity is not sufficient for induced cell movement. J Cell Biol 1994; 127:847-857.
6. Gerhardt H, Golding M, Fruttiger M, Ruhrberg C, Lundkvist A, Abramsson A, *et al*. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. J Cell Biol 2003; 161:1163-1177.
7. Zheng Y, Zhang C, Croucher DR, Soliman MA, St-Denis N, Pasculescu A, *et al*. Temporal regulation of EGF signalling networks by the scaffold protein Shc1. Nature 2013; 499:166-171.
8. Giese A, Loo MA, Tran N, Haskett D, Coons SW, Berens ME. Dichotomy of astrocytoma migration and proliferation. Int J Cancer 1996; 67:275-282.
9. Castoria G, Lombardi M, Barone MV, Bilancio A, Di Domenico M, Bottero D, *et al*. Androgen-stimulated DNA synthesis and cytoskeletal changes in fibroblasts by a non-transcriptional receptor action. J Cell Biol. 2003; 161:547-556.
10. De Donatis A, Comito G, Buricchi F, Vinci MC, Parenti A, Caselli A, *et al*. Proliferation versus migration in platelet-derived growth factor signaling: the key role of endocytosis. J Biol Chem 2008; 283:19948-19956.
11. Niu Y, Chang TM, Yeh S, Ma WL, Wang YZ, Chang C. Differential androgen receptor signals in different cells explain why androgen-deprivation therapy of prostate cancer fails. Oncogene 2010; 29:3593-3604.
12. Sharpe RM. Pathways of endocrine disruption during male sexual differentiation and masculinization. Best Pract Res Clin Endocrinol Metab 2006; 20:91-110.
13. Montgomery RB, Mostaghel EA, Vessella R, Hess DL, Kalhorn TF, Higano CS, *et al*. Maintenance of intratumoral androgens in metastatic prostate cancer: a mechanism for castration-resistant tumor growth. Cancer Res 2008; 68:4474-4454.
14. Okitsu K, Kanda T, Imazeki F, Yonemitsu Y, Ray RB, Chang C, *et al*. Involvement of interleukin-6 and androgen receptor signaling in pancreatic cancer. Genes Cancer 2010; 1:859-867.
15. Castoria G, Giovannelli P, Di Donato M, Ciociola A, Hayashi R, Bernal F, *et al*. Role of non-genomic androgen signalling in suppressing proliferation of fibroblasts and fibrosarcoma cells. Cell Death Dis 2014; 5:e1548.
16. Castoria G, D'Amato L, Ciociola A, Giovannelli P, Giraldi T, Sepe L, *et al*. Androgen-induced cell migration: role of androgen receptor/filamin A association. PLoS One 2011; 6:e17218.
17. Diaw L, Roth M, Schwinn DA, d'Alelio ME, Green LJ, Tangrea JA. Characteristics of a human prostate stromal cell line related to its use in a stromal-epithelial coculture model for the study of cancer chemoprevention. In Vitro Cell Dev Biol Anim 2005; 41:142-148.
18. Sun X, He H, Xie Z, Qian W, Zhau HE, Chung LW, *et al*. Matched pairs of human prostate stromal cells display differential tropic effects on LNCaP prostate cancer cells. In Vitro Cell Dev Biol Anim 2010; 46:538-546.
19. Singh M, Jha R, Melamed J, Shapiro E, Hayward SW, Lee P. Stromal Androgen Receptor in Prostate Development and Cancer. Am J Pathol 2014; 184:2598-2607.
20. Castoria G, Migliaccio A, Di Domenico M, Lombardi M, de Falco A, Varricchio L, *et al*. Role of atypical PKC in estradiol-triggered G1/S progression of MCF-7 cells. Mol Cell Biol 2004; 24:7643-7653.
21. Castoria G, Migliaccio A, Bilancio A, Di Domenico M, de Falco A, Lombardi M, *et al*. PI3-kinase in concert with Src promotes the S-phase entry of estradiol-stimulated MCF-7 cells. EMBO J 2001; 20:6050-6059.
22. Migliaccio A, Castoria G, Di Domenico M, de Falco A, Bilancio A, Lombardi M, *et al*. Steroid-induced androgen receptor-oestradiol receptor β-Src complex triggers prostate cancer cell proliferation. EMBO J 2000; 19:5406-5417.
23. Le Romancer M, Treilleux I, Leconte N, Robin-Lespinasse Y, Sentis S, Bouchekioua-Bouzaghou K, *et al*. Regulation of estrogen rapid signaling through arginine methylation by PRMT1. Mol Cell 2008; 31:212-221.
24. Cabodi S, Moro L, Baj G, Smeriglio M, Di Stefano P, Gippone S, *et al*. p130Cas interacts with estrogen receptor alpha and modulates non-genomic estrogen signaling in breast cancer cells. J Cell Sci 2004; 117:1603-1611.
25. Fernando RI, Wimalasena J. Estradiol abrogates apoptosis in

breast cancer cells through inactivation of BAD: Ras-dependent nongenomic pathways requiring signaling through ERK and Akt. Mol Biol Cell 2004; 15:3266-3284.

26. Hisamoto K, Ohmichi M, Kurachi H, Hayakawa J, Kanda Y, Nishio Y, *et al*. Estrogen induces the Akt-dependent activation of endothelial nitric-oxide synthase in vascular endothelial cells. J Biol Chem 2001; 276:3459-3467.
27. Poulard C, Treilleux I, Lavergne E, Bouchekioua-Bouzaghou K, Goddard-Léon S, Chabaud S, *et al*. Activation of rapid oestrogen signalling in aggressive human breast cancers. EMBO Mol Med 2012; 4:1200-1213.
28. Vicent GP, Nacht AS, Zaurín R, Ballaré C, Clausell J, Beato M. Minireview: role of kinases and chromatin remodeling in progesterone signaling to chromatin. Mol Endocrinol 2010; 24:2088-2098.
29. Hammes SR, Levin ER. Minireview: Recent advances in extranuclear steroid receptor actions. Endocrinology 2011; 152:4489-4495.
30. Giovannelli P, Di Donato M, Giraldi T, Migliaccio A, Castoria G, Auricchio F. Targeting rapid action of sex-steroid receptors in breast and prostate cancers. Front Biosci (Elite Ed) 2012; 4:453-461.
31. Migliaccio A, Varricchio L, De Falco A, Castoria G, Arra C, Yamaguchi H, *et al*. Inhibition of the SH3 domain-mediated binding of Src to the androgen receptor and its effect on tumor growth. Oncogene 2007; 26:6619-6629.
32. Varricchio L, Migliaccio A, Castoria G, Yamaguchi H, de Falco A, Di Domenico M, *et al*. Inhibition of Estradiol Receptor/Src Association and Cell Growth by an Estradiol Receptor {alpha} Tyrosine-Phosphorylated Peptide. Mol Cancer Res 2007; 11:1213-1221.
33. Zhou AX, Hartwig JH, Akyürek LM. Filamins in cell signaling, transcription and organ development. Trends Cell Biol 2010; 20:113-123.
34. Ozanne DM, Brady ME, Cook S, Gaughan L, Neal DE, Robson CN. Androgen receptor nuclear translocation is facilitated by the f-actin cross-linking protein filamin. Mol Endocrinol 2000; 14:1618-1626.
35. Loy CJ, Sim KS, Yong EL. Filamin-A fragment localizes to the nucleus to regulate androgen receptor and coactivator functions. Proc Natl Acad Sci USA 2003; 100:4562-4567.
36. Wang Y, Kreisberg JI, Bedolla RG, Mikhailova M, deVere White RW, Ghosh PM. A 90 kDa fragment of filamin A promotes Casodex-induced growth inhibition in Casodex-resistant androgen receptor positive C4-2 prostate cancer cells. Oncogene 2007; 26:6061-6070.
37. Anderson R, Fässler R, Georges-Labouesse E, Hynes RO, Bader BL, Kreidberg JA, *et al*. Mouse primordial germ cells lacking beta1 integrins enter the germline but fail to migrate normally to the gonads. Development 1999; 126:1655-1664.
38. Sato T, Matsumoto T, Kawano H, Watanabe T, Uematsu Y, Sekine K, *et al*. Brain masculinization requires androgen receptor function. Proc Natl Acad Sci USA 2004; 101:1673-1678.
39. Bedolla RG, Wang Y, Asuncion A, Chamie K, Siddiqui S, Mudryj MM, *et al*. Nuclear versus cytoplasmic localization of filamin A in prostate cancer: immunohistochemical correlation with metastases. Clin Cancer Res 2009; 15:788-796.
40. Mooso BA, Vinall RL, Tepper CG, Savoy RM, Cheung JP, Singh S, *et al*. Enhancing the effectiveness of androgen deprivation in prostate cancer by inducing Filamin A nuclear localization. Endocr Relat Cancer 2012; 19:759-777.
41. Castoria G, Giovannelli P, Di Donato M, Hayashi R, Arra C, Appella E, *et al*. Targeting androgen receptor/Src complex impairs the aggressive phenotype of human fibrosarcoma cells. PLoS One 2013; 8:e76899.
42. Becker W. Emerging role of DYRK family protein kinases as regulators of protein stability in cell cycle control. Cell Cycle 2012; 11:3389-3394.
43. Deng X, Ewton DZ, Pawlikowski B, Maimone M, Friedman E. Mirk/dyrk1B is a Rho-induced kinase active in skeletal muscle differentiation. J Biol Chem 2003; 278:41347-41354.
44. Deng X, Friedman E. Mirk kinase inhibition blocks the in vivo growth of pancreatic cancer cells. Genes Cancer 2014; 5:337-347.
45. Jin K, Park S, Ewton DZ, Friedman E. The survival kinase Mirk/Dyrk1B is a downstream effector of oncogenic K-ras in pancreatic cancer. Cancer Res 2007; 1:7247-7255.
46. Lauth M, Bergström A, Shimokawa T, Tostar U, Jin Q, Fendrich V, *et al*. DYRK1B-dependent autocrine-to-paracrine shift of Hedgehog signaling by mutant RAS. Nat Struct Mol Biol 2010; 17:718-725.
47. Guerrini A, Tesei A, Ferroni C, Paganelli G, Zamagni A, Carloni S, *et al*. A new avenue toward androgen receptor pan-antagonists: C2 sterically hindered substitution of hydroxy-propanamides. J Med Chem 2014; 57:7263-7279.