Novel insights on imaging sex hormone-dependent tumourigenesis *in vivo*

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Abstract

Sex hormones modulate proliferation, apoptosis, migration, metastasis and angiogenesis in cancer cells influencing tumourigenesis from the early hyperplastic growth till the end-stage metastasis. Although decades of studies have detailed these effects at the level of molecular pathways, where and when these actions are needed for the growth and progression of hormone-dependent neoplasia is poorly elucidated. Investigation of the hormone influences in carcinogenesis in the *spatio*-temporal dimension is expected to unravel critical steps in tumour progression and in the onset of resistance to hormone therapies. Non-invasive *in vivo* imaging represents a powerful tool to follow in time hormone signalling in the whole body during tumour development. This review summarizes the tools currently available to follow hormone action in living organisms.

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Introduction

Neoplastic transformation is a highly regulated and ordered process, where a *sequela* of biological events leads cancer cells to acquire specific phenotypic traits necessary to escape the strong selective pressure of the host tumour surveillance (Merlo *et al.* 2006). In the current view, cancer development is more related to the coordinated generation of a new tissue rather than to the mere disruption of a biological program; the vast network of intracellular and extracellular signals involved in carcinogenesis are well coordinated in time and are characterized by several discrete steps leading to the acquisition of specific cell functions, which are commonly observed during cell transformation (Hanahan & Weinberg 2000). The attempts to decode the order and timing for the genetic changes typically associated with carcinogenesis were only partially successful; this led to formulate the hypothesis that the process is redundant involving a large variety of epigenetic/genetic mutations occurring in oncogenes/oncosuppressors (Esteller 2006), which cannot be directly associated to specific tumour stages

(Hanahan & Weinberg 2000). In hormone-related carcinogenesis, endogenous and exogenous hormones influence a multiplicity of cell functions. Dysregulation of sex hormone-receptor signalling occurs in the tumourigenesis of breast, endometrial, ovary, prostate and testis, where oestrogens, progestin and androgens have been shown to modulate the proliferation of epithelial cells, thus increasing the probability of accumulation of genetic errors. Contribution of sex hormones to the malignant phenotype, besides the induction of the initial hyperplasia, is still elusive. A multidisciplinary approach combining *in vivo* imaging with system biology is expected to add novel insights on the temporal characterization of the changes stimulated by the hormones, leading to the progression of hormone-related cancers.

The influence of steroid hormone action on tumour growth and progression

Sex hormones such as oestrogens, progestins and androgens are hydrophobic ligands, which bind to

transcription factors belonging to the superfamily of intracellular receptors (IRs). These receptors can be activated by the cognate ligand or in its absence, by post-translational modifications elicited through the intracellular signalling of membrane receptors (Weigel & Moore 2007, Stanisić *et al.* 2010). Upon ligand binding, receptor activation occurs via diversified pathways involving genomic or non-genomic mechanisms (Migliaccio *et al.* 2007), i.e. the activated receptor may directly bind to the DNA-responsive elements in the regulatory regions of these genes or may influence other pathways involved in cell proliferation by interfering with specific proteins in the cytoplasm (e.g. AKT/PI3K) or in the nucleus (e.g. NFkB, AP-1 and SP-1). Co-activators, co-repressors and integrators interact with IRs to mediate their transcriptional activity; the expression and activity of these co-regulators may be tissue-specific and in turn can be modulated by cell metabolism. In the whole organism, co-regulators integrate positional information with signal transduction to produce in each cell a selective modulation of steroid receptor target genes. In some target cell, including endocrine-related tumour cells, the activated receptor is able to stimulate G1/S-phase transition through the G1 restriction point by inducing the expression of specific cell cycle regulators, such as c-myc, c-fos, c-jun and cyclinD1 (Butt *et al.* 2005, Lamont & Tindall 2010). Stimulation of these mitogenic pathways eventually promotes the hyperplastic growth of epithelial cells in reproductive tissues; however, this proliferative induction does not entail for the full carcinogenic potential of a dysregulated steroid hormone signal: indeed, steroid hormones modulate apoptosis, promote migration, metastasis and angiogenesis functions in tumour cells (Kaarbø *et al.* 2007, Lewis-Wambi & Jordan 2009, Sarker *et al.* 2009, Dondi *et al.* 2010). Current hypothesis propose that the influence of hormones extends throughout the carcinogenesis process. In clinical experience, this is well exemplified by the fact that anti-hormone drugs are active at different stages preventing the early tumour onset (William *et al.* 2009) or metastasis formation (Boccardo *et al.* 1999, Howell *et al.* 2005). How the hormone signalling is integrated in the process of carcinogenesis and which is the contribution to the generation of the transformed phenotype is only partially elucidated; it may be expected that non-invasive *in vivo* imaging tools allowing the investigation of molecular events in time might help filling this gap. This review aims at illustrating the *in vivo* imaging methodologies available now (supplementary data, see section on supplementary data given at the end of this article)

and applicable to the measurement in real time, of: i) receptor expression, ii) hormone production, iii) receptor activation and iv) receptor-dependent modulation of specific cellular pathways.

Molecular imaging of steroid receptor expression

Regulation of steroid receptor expression is governed by complex mechanisms (Stanisić *et al.* 2010), including usage of multiple promoters (Sasaki *et al.* 2003), generation of different splicing variants, regulation of mRNA stability (Hirata *et al.* 2003) and receptor proteolysis (Alarid 2006). These mechanisms were found, in some case, to be deregulated in tumour cells, although the existence of a coordinated action modulating the receptor expression during hormonal tumourigenesis has not been investigated. The dynamic view offered by imaging provides a unique insight on the interrelation between hormone receptor regulation and tumour insurgence and progression. This might contribute, for example, to gain information on the mechanisms underlying the arousal of hormone-resistant subclones in patients (a tumour evolution that worsen prognosis and invariably occurs in chronic treatments with hormonal therapy). In a significant proportion of such patients, for unknown reasons, a down-regulation of receptor expression is observed (Kuukasjärvi *et al.* 1996, Chen *et al.* 2004, Sabnis *et al.* 2008, Musgrove & Sutherland 2009, Zilli *et al.* 2009). Imaging gives the possibility to isolate tumours at a precise stage when this event occurs, and hence allows investigating which signalling pathways are directly linked to receptor down-regulation. Steroid receptor expression in tumours is measurable with positron emission tomography (PET) and single photon emission computed tomography (SPECT) (supplementary data, see section on supplementary data given at the end of this article) using oestrogens, progestins and androgens chemically labelled with ⁷⁷Br (Katzenellenbogen *et al.* 1981, McElvany *et al.* 1982), ¹²³I (Zielinski *et al.* 1989, Rijks *et al.* 1998), ¹⁸F (Kiesewetter *et al.* 1984, Liu *et al.* 1992, Katzenellenbogen *et al.* 1997, Jonson & Welch 1998) radioisotopes (for a review on nuclear imaging see de Vries *et al.* (2007) and Hospers *et al.* (2008)). PET analysis provides an accurate measurement of receptor expression that correlates with the more classical immunohistochemical-based quantification of receptor content (Peterson *et al.* 2008). These radiotracers have been largely applied in clinics particularly to the selection of patients with a chance to respond to hormonal therapy (for a review see Dunphy & Lewis

(2009)); however, their use to study the molecular basis of hormone dependency of endocrine tumour model have not yet been exploited; it has to be considered the facts that these methodologies normally require expensive instrumentation and, most of the time, also a cyclotron, therefore are restricted to a relatively small number of laboratories. Recently, Cerenkov radiation imaging was presented as a novel concept, allowing the use of conventional optical imaging devices for the detection of radiotracers, thus opening new perspective for the *in vivo* detection of radiolabelled hormones (Robertson *et al.* 2009, Hu *et al.* 2010, Ruggiero *et al.* 2010, Spinelli *et al.* 2010).

Molecular imaging of hormone production

In the central nervous system as well as in the periphery, multiple mechanisms govern the production and the metabolism of steroid hormones. Dysregulation of this network of signalling pathways contributes to endocrine tumourigenesis; indeed, drugs that target steroid hormone synthesis or metabolism are among the most efficacious for the treatment of hormone-dependent breast and prostate cancers (e.g. aromatase inhibitors and GnRH analogues). Hormones, metabolites and catabolites are able to bind and activate different types of receptors that fine-tune the homeostasis of target tissues. A well-characterized example is the prostate tissue, where a network of hormone signals controls the tissue physiology and prevents tumourigenesis. In the prostate, dihydrotestosterone (DHT) controls the physiological proliferation, while dysregulation of this signal eventually promotes prostate cancer cell growth. The inactive androgen, testosterone, is either reduced to the active form DHT or aromatized to 17 β -oestradiol (E₂); DHT and E₂ bind androgen receptor (AR) and oestrogen receptor (ER)s respectively, with different and sometime opposing effects on the prostate target cells (Bilińska *et al.* 2006, Carruba 2007, Ellem & Risbridger 2010). Furthermore, DHT can also be reduced to 5 α -androstane-3 β ,17 β -diol (3 β -Adiol), a metabolite which binds and activate preferentially the ER β isoform (Imamov *et al.* 2004, Guerini *et al.* 2005), but not AR, counteracting DHT action on the growth of the normal prostate (Weihua *et al.* 2002) and on the proliferation, migration and metastasis of prostate cancer cells (Weihua *et al.* 2002, Dondi *et al.* 2010). When and how perturbation of this complex hormone balance occurs during prostate tumourigenesis is not easy to address experimentally. We need to develop appropriate tools to study the dynamics of ligand or metabolite production in the whole organism. Imaging the activity of specific

enzymes involved in steroidogenesis or the presence of certain hormones or their metabolites in a tissue could be very helpful to dissect temporarily each event. Some of the current methodologies based on reporter systems may be adapted to the measurement of the synthesis of ligands for a given steroid receptor *in vivo*. Two types of sensors were developed to measure the hormone production *in vivo* and both rely to the ability of the ligand to bind and transcriptionally activate the steroid receptor: i) genetic two-hybrid system, where the receptor 'ligand-binding domain' (LBD) is fused to the Gal4 DNA binding domain and ii) an intramolecular folding system based on reporter genes fused to the receptor LBD. The two-hybrid system strategy is based on the ligand-dependent activation of the Gal4–LBD fusion protein, which in turn induces the transcription of a reporter gene (e.g. β -galactosidase) driven by a GAL4-responsive promoter; in this system, the amount of reporter protein synthesized is directly proportional to the hormone contents in a given tissue (Mata de Urquiza & Perlmann 2003). Transgenic reporter mice generated with these types of biosensors were demonstrated to be useful tools to investigate the production of endogenous retinoids or thyroid hormones during embryo development and in mature mice (Solomin *et al.* 1998, de Urquiza *et al.* 2000, Quignodon *et al.* 2004). The second strategy is based on a fusion protein containing the hormone receptor LBD which splits luciferase polypeptide in two parts; after the hormone binding, an intramolecular folding occurs and reconstitutes the luciferase activity. These systems were proven to be helpful for measuring the activity of selective oestrogen receptor modulator in cell lines (Paulmurugan & Gambhir 2006, Paulmurugan *et al.* 2009). These sensors, if expressed ubiquitously with appropriate reporter genes (Weissleder & Pittet 2008), allow for the *in vivo* imaging of hormone production.

Molecular imaging of steroid receptor activity

A major limitation in the studies measuring ligand and/or receptor distribution within the body is the lack of any insight on the extent to which the receptor is activated and contributes to the cell phenotype. Indeed, the amount of hormones in the blood does not have a prognostic value in clinic and is usually not indicative of the state of the activity of the receptor in the tumour; mechanisms such as receptor desensitization, ligand-independent activation and tissue-specific interaction with co-regulators are well known to strongly modulate sex-steroid signalling pathways. Thus, independent of

hormone levels or receptor expression, it would be important to identify the time points when the receptor activity is actually required or lost during endocrine-related cancer progression; this information can be provided by *in vivo* imaging. A number of systems have been developed for measuring steroid-receptor activity *in vivo*, of which some of them were specifically designed for *in vivo* imaging. Since sex-steroid receptors are transcription factors, receptor activation is usually measured for its ability to induce the transcription of a reporter gene. Reporter systems of this type were applied to the production of transgenic reporter mice successfully obtained by our group (Ciana et al. 2001) and other laboratories (Lemmen et al. 2004, Hsieh et al. 2005), thanks to the use of an appropriate technology to prevent the position effects linked to the transgenesis procedure (Maggi et al. 2004). In these models, it is possible to measure the state of ER and AR activation in all mouse tissues. Bioluminescence imaging (supplementary data, see section on supplementary data given at the end of this article) applied to these reporter mice

allowed the measurement of receptor activation in physiology (Ciana et al. 2003, 2005, Lemmen et al. 2004) and in cancer biology (Fig. 1, Lyons et al. 2006, Hsieh et al. 2007); in these works, steroid receptor activation was evaluated in the tumours of living mice, and was used as a marker to identify those animals that after hormone ablation were capable of sustaining tumour growth. Another strategy to measure receptor activity *in vivo* was developed by O'Malley's group that generated the so called 'indicator' mouse models, allowing the measurement of receptor expression and activity simultaneously, *in vivo* (Han et al. 2005, 2009, Ye et al. 2005). 'Indicators' are mice genetically engineered with bacterial artificial chromosome (BAC) which carries a genomic region of a nuclear receptor (ER, AR or PR) with the inserted GAL4 DNA-binding domain and encoding for a fusion protein (ER-GAL4, AR-GAL4 or PR-GAL4) that displays the same tissue distribution of the endogenous receptor; in addition, each BAC also carries a reporter system for the nuclear receptor activity. The receptor/GAL4 fusion protein once expressed becomes activated and binds to the

Figure 1 Bioluminescence *in vivo* imaging of breast cancer growth in mice. (A) Pictures represent a non-invasive bioluminescence imaging experiment carried out with an ERE-Luc reporter mouse treated with an oral carcinogen, 7,12-dimethylbenz(a)anthracene, following a classical mammary carcinogenesis protocol in mice. The red oval shows the breast area, in which ER activity is growing by the time, visible already at week 4 and then very prominent at week 8. Mice received an i.p. injection of 50 mg/kg D-luciferin (Promega) 20 min before bioluminescence quantification, to achieve a uniform biodistribution of the substrate; imaging was carried out with a Night Owl imaging unit (Berthold Technologies, Bad Wildbad, Germany), consisting of a Peltier-cooled charge-coupled device slowscan camera equipped with a 25 mm, f/0.95 lens. Pictures were generated by a Night Owl LB981 image processor and transferred via video cable to a peripheral component interconnect frame grabber using WinLight32 software (Berthold Technologies). (B) *Ex vivo* imaging of the explanted breast performed at week 8 shows the ER activity (photon emission) of the breast lesion evident in the mammary gland fat pad. (C) Quantification of photon emission from the breast area of the mouse. Photon emission was integrated over a period of 5 min; light emission is expressed as number of counts/cm² per second.

promoter of the reporter system, inducing the production of a green fluorescence protein (GFP). Thus, GFP level in the indicator mouse is proportional to the receptor expression and activation at the same time. Unfortunately, the GFP used in the generation of these models is not an ideal reporter for *in vivo* imaging (mainly due to the wide overlapping of the peak of the fluorescence emitted by the GFP and the autofluorescence peak naturally present in the tissues). Also in this case, the appropriate choice of the reporter gene used in the indicator mouse would allow the *in vivo* imaging of receptor expression and activity.

Molecular imaging of signalling pathways activated by the hormone

Liganded or unliganded modulation of the steroid receptor results in the activation or inhibition of intracellular circuitry translating the hormonal message into phenotypic effects. Sex-steroid receptor activation in hormone-dependent neoplasia modulates a broad range of key pathways for tumorigenesis, including those governing apoptosis, proliferation and angiogenesis.

Apoptosis

In the war against cancer, one of the major problems arises from the changes of the tumour phenotype in terms of sensitivity to proapoptotic treatments (Shankaranarayanan *et al.* 2009). In hormone-dependent tumours, sex-steroid receptors are known to differentially regulate apoptotic pathways depending on the tumour stage or the cellular context (Lewis-Wambi & Jordan 2009); *in vivo* imaging could help to identify the tumour stage at which this modulation occurs and to correlate it with the sensitivity of the cells to apoptotic stimuli. A wide array of non-invasive imaging techniques has been developed so far to follow the molecular events specifically occurring during apoptosis. It is possible, for example, to measure caspases activation by using luminescent, fluorescent or radio-labelled substrates carrying a caspase recognition sequence (e.g. Asp-Glu-Val-Asp: DEVD) or to measure expression of annexin V apoptotic marker with fluorescent/radioactive probes. Most of these imaging tools are currently used in the development of new anti-cancer treatment and in clinical studies to assess treatment responses (Kurihara *et al.* 2008).

Proliferation

The mechanism underlying stimulation of cancer cell proliferation by sex hormones has been investigated to

the molecular details in about four decades of research in animals and cultured cells. However, there are preclinical (Zhao *et al.* 1992) and clinical data (Clarke *et al.* 1997, Anderson 2002, Clarke 2004) challenging the simplest view that hormones are always behaving as inducer of cell proliferation. These studies suggest that, during tumour development, the sensitivities of neoplastic cells to the hormones vary in terms of proliferative response; thus, it remains unclear whether the proliferative effects of the hormone is required during specific stages or throughout whole tumour progression. Non-invasive *in vivo* imaging may provide novel insights into this issue allowing to follow the proliferative activity of every single tumour during its growth and metastasis and to evaluate its sensitivity to hormones at every different stage. Nuclear, magnetic resonance and optical imaging tools are used to measure cell proliferation *in vivo*. Nuclear imaging of proliferation is based on probes designed to measure in cancer cells: i) the accelerated DNA synthesis, e.g. nucleotide analogues like [11C-methyl]TdR, 2'-deoxy-2'-fluoro-1-β-D-arabinofuranosyl-thymine (FMAU), FLT (1-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl-uracil)-bromouracil (FBAU), [18F]fluorothymine) or ii) the accelerated cellular metabolism, e.g. labelled sugar, 18F-fluorodeoxyglucose (FDG), amino acid (L-[11C-methyl]-methionine and L-[1-11C]tyrosine ([18F]-fluorocholine) or lipids ([18F]-fluoroethylcholine and [11C]-choline). PET evaluation of uncontrolled proliferation in hormone-sensitive breast cancers was applied to evaluate their response to chemotherapy (Sun *et al.* 2005, Pio *et al.* 2006, Kenny *et al.* 2007, Kim *et al.* 2007) and for tumour staging (Kwock *et al.* 2006, Laprie *et al.* 2008). Optical imaging tools, including molecular probes (selectively binding the tumour cells) or reporter genes were developed to label tumour growth and are currently used in preclinical studies to localize as little as 100–1000 xenografted cancer cells in animal models; these systems provide a quantitative measure of cancer growth and metastasis and hence found useful for screening compounds with anti-cancer properties. Some reporter system was also applied to the generation of transgenic reporter mice in which it is possible to directly measure mitogenic pathways in the animal tissues. Uhrbom *et al.* 2004 have generated a transgenic mouse expressing luciferase only when the Rb pathway is inactivated and the transformed cells underwent uncontrolled proliferation; activation of this biosensor allows the detection of sporadic tumour arousal and to follow the neoplastic growth in time in the whole body by using bioluminescence *in vivo* imaging. A further refinement of this model refers to

a recently generated transgenic reporter mouse, in which luciferase is a measure of all mitogenic signals in the body (Piaggio G, Maggi A, Ciana P, manuscript in preparation). In this mouse model, it is possible to detect proliferation not only in the growing tumour but also in pre-malignant lesion and in the normal tissues where proliferation occurs as a physiological response to tumour growth (e.g. immune system responses, vessel formation and stroma cells responses). This model can also be particularly relevant in drug development to evaluate toxicity of compounds interfering with the normal homeostatic proliferation found in body tissues (e.g. bone marrow toxicity).

Angiogenesis

When cancer grows beyond 1–2 mm³ in diameter, there is a requirement for new blood vessels to supply nutrients and oxygen. For endocrine cancers, it is still largely debated whether sex hormones have a role in this neo-angiogenic process; indeed, there are evidence for a direct transcriptional control of the hormones on vascular endothelial growth factor (VEGF) and $\alpha_v\beta_3$ integrin genes, two key players in neo-angiogenesis (Bogin & Degani 2002, Buteau-Lozano *et al.* 2002, Hood & Cheresh 2002). However, no mechanistic hypothesis into this hormone control has been proposed yet. It would be interesting, for example, to evaluate the role of endocrine signals on the mobilization, recruitment and differentiation of endothelial progenitor cells (bone marrow-derived or resident) and on the subsequent new vessel formation. Although some system have been devised to label progenitor stem cells for imaging their fate in the body (Schroeder 2008) and to evaluate the associated neo-angiogenesis, to the best of our knowledge, there has been no report applying imaging technology for the characterization of sex hormone signalling on new vessel formation. Several imaging modalities can be used to visualize neo-angiogenesis with probes detecting VEGF or $\alpha_v\beta_3$ integrins expression, e.g. PET imaging with radiolabelled antibodies against VEGF (Nagengast *et al.* 2007) or ultrasound imaging of VEGF/VEGF monoclonal antibody conjugated with microbubbles (Korpanty *et al.* 2007, Willmann *et al.* 2008). Since $\alpha_v\beta_3$ integrins recognize specific component of the extracellular matrix containing the arginine-glycine-aspartic acid (RGD), RGD-containing peptides have also been developed as targeting ligands for imaging neo-vasculature formation in tumours; indeed, radiolabelled RGD-containing compounds such as (¹⁸F)-Galacto-RGD or (^{99m}Tc)-NC100692 have been successfully used for

PET analysis of the neo-angiogenesis associated to squamous cell carcinoma or breast cancer (Bach-Gansmo *et al.* 2006, Beer *et al.* 2007). Bioluminescence modality is also often applied for the detection of new vessel formation (Fukumura *et al.* 1998, Snoeks *et al.* 2010); in this research line, an interesting transgenic mouse model was recently developed as a tool to follow the activation of the hypoxia-inducible factor1 α (Hif1 α). Hif1 α is a transcription factor turning on the neo-angiogenesis programme when cells are under low-oxygen conditions. In this work, Safran *et al.* reported the generation of a reporter mouse ubiquitously expressing a bioluminescent reporter consisting of the firefly luciferase fused to a region of Hif1 α that is sufficient for oxygen-dependent degradation. In all tissues of this mouse, any hypoxic condition induces an increased bioluminescent emission, which can be detected by optical imaging (Safran *et al.* 2006).

Conclusions and perspectives

There is only a limited knowledge on the dynamic influence of sex steroid hormones on the development and progression of endocrine-related cancers. New tools, which would allow the investigation of molecular events in the *spatio*-temporal dimension, are required to follow the steroid hormone message from its circulation in the body till the activation of intracellular signalling pathways in the tumour itself and in the normal tissues. *In vivo* imaging comprises a cluster of technologies allowing the measurement of biological events with respect to time and in the whole animal, information that is particularly relevant for hormonal carcinogenesis also in consideration of the systemic effects of hormones and the complex network of signals going back-forward from CNS to the reproductive tissues. Each different imaging modality presents *pros* and *cons* which currently limits the analysis in terms of sensitivity, resolution and type of information provided on the biological events (Massoud & Gambhir 2003; supplementary data, see section on supplementary data given at the end of this article). To overcome these limitations, a wealth of novel systems and novel instrumentations allowing the integration of different imaging modalities (Stell *et al.* 2007a, Tian *et al.* 2008, Lee & Chen 2009) have been proposed, including PET/CT (Cherry 2009), PET/fluorescence/bioluminescence (Kesarwala *et al.* 2006), PET/MRI (Sauter *et al.* 2010) and other combinations of technology, which integrate molecular information with morphological information and uncouple *spatial* resolution with higher degree of

sensitivity and deep penetration through body tissues. Further advancement in the field is also expected in the way probes and biosensors are designed to widen the list of molecular events that can be monitored by *in vivo* imaging (Stell *et al.* 2007b, Pysz *et al.* 2010). Among them, fluorescence resonance energy transfer (FRET) and bioluminescence resonance energy transfer (BRET) are promising technologies which have already opened up new avenues of measuring protein–protein and ligand–receptor interactions, membrane receptor activation and calcium signalling. Several methodologies based on FRET/BRET biosensors have been applied to the study of the key components of the hormone signalling, including ligand binding (Michellini *et al.* 2004, De *et al.* 2005), receptor dimerization (Schaufele *et al.* 2005, Powell & Xu 2008) and recruitment of co-regulators (Koterba & Rowan 2006, Ozers *et al.* 2007). These methods have not been fully developed for the study of sex hormone signal *in vivo*; however, we expect that BRET and FRET, when combined with intravital microscopy or optical detection systems, will significantly improve the *spatio*-temporal analysis of hormonal pathways from single cell to a whole body resolution. More information are also expected to come from the application of imaging onto cancer stem cell field that already gave promising results (Hong *et al.* 2010); imaging might help answering several open questions about the role of sex hormones on the fate of cancer stem cells (LaMarca & Rosen 2008). This is a rather important issue, which needs clarification to understand the mechanisms underlying the early events occurring during hormonal transformation and in the establishment of resistance.

Obviously, the information provided by *in vivo* imaging alone cannot be sufficient to generate a comprehensive representation of the molecular pathways involved in tumour development; in this sense, *in vivo* imaging represents a good way to identify tumour stages at which changes in selected molecular pathways (e.g. sex-hormone receptor signals) occur, before they produce evident consequences on the phenotype of transformed cell. Imaging analysis of different aspects of the steroid hormone signalling is now expected to drive classical molecular genetics and genomic studies towards the characterization of specific steps during carcinogenesis, when the genetic reprogramming actually occurs. Hopefully, the description of the molecular pathways involved in these steps will help identifying unique marker signatures of neoplasia progression and inspire the design of new therapeutic strategies for hormone-related cancers and to overcome the problem of drug resistance.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/ERC-10-0332>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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