PROJECT FINAL REPORT

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[2] The home page of the website should contain the generic European flag and the FP7 logo which are available in electronic format at the Europa website (logo of the European flag: http://europa.eu/abc/symbols/emblem/index_en.htm ; logo of the 7th FP: http://ec.europa.eu/research/fp7/index_en.cfm?pg=logos). The area of activity of the project should also be mentioned.

Final publishable summary report

The growth of tumours at distant sites (metastasis) is the process responsible for over 90% of cancer deaths and there is considerable need for a better understanding of this process and for new therapies for metastatic cancer. The SFMET project focussed on two proteins (the growth/motility factor HGF/SF and its membrane receptor MET) that can cause cancer cells to move at a distance from their primary site and thus play an important role in the early stages of metastasis.

1. Aims

The SFMET project had three main aims:

1.1 To understand how HGF/SF and MET contribute to the process of tumour invasion and metastasis by addressing two key questions, namely how the conditions of low oxygen tension (hypoxia) typical of growing tumours can cause activation of HGF/SF and MET and hence metastasis (WP1) and how HGF/SF and MET cooperate with two other signalling systems, WNT and the chemokines-chemokine receptors, in promoting invasion and metastasis (WP2).

1.2. To determine crystal structures of the HGF/SF-MET complex (WP3) and of suitably engineered fragments of HGF/SF, such as NK1, that can compete with the natural MET ligand HGF/SF and thus block HGF/SF-dependent MET signalling in cancer cells (WP4).

1.3. To set the foundations for the development of three new classes of low molecular weight (drug-like) inhibitors of MET signalling, namely compounds that may bind NK1 or MET (WP5) or SHP-2, a key downstream effector of HGF/SF and MET (WP6).

2. Results

The SFMET project has achieved all its initial aims and, in three crucial areas, it has exceeded the initial work plan and generated additional and valuable information on the structure/function of HGFSF and rational strategies for inhibiting HGF/SF and MET in cancer. An outline of the achievements of the individual WP is given below:

2.1 Work package 1. Hypoxia, MET and tumour invasion. (Paolo Comoglio, IRCC, Candiolo Italy)

Expression of the c-MET gene is regulated by hypoxia and this regulation may explain the increased invasiveness of hypoxic tumours. The major objective of WP1 was to develop

mouse models of tumour hypoxia in order to further define the role of hypoxia-induced MET expression in tumor invasion and metastasis in vivo. Another objective of WP1 was to test whether specific inhibitors of HGF/MET can effectively interfere with hypoxia-induced tumour invasion in the above in vivo models. Both goals have been successfully achieved, as detailed below.

In order to study the role of hypoxia in tumour invasion, experimental tumours with different degrees of tumor hypoxia were produced and tumour angiogenesis, proliferative and apoptotic indexes, local invasion, distant metastases as well as MET expression and activation were measured.

To increase tumour hypoxia, animals bearing experimental tumors were treated with various angiogenesis inhibitors. This resulted in enhanced MET expression and activation and dramatically boosted both local and systemic tumour dissemination but, remarkably, interestingl, the tumour invasion induced by anti-angiogenic drugs could be prevented by combined administration of a specific MET kinase inhibitor, thus confirming MET as a fundamental mediator of hypoxia-promoted metastasis. Conversely, experimental manipulations that increased the level of oxygen within the tumour mass, for example as a result of forced expression of the oxygen transporter myoglobi, displayed an enhanced ability to maintain an aerobic metabolism under hypoxia, retained lower levels of HIF-1α compared to control cells and displayed a more differentiated phenotype and reduced local invasion and metastasis.

Together, these results demonstrate that hypoxia is a driving force of tumour invasion and progression, and that MET is a fundamental mediator of this force. They also imply that targeting MET with specific pharmacological agents represents a valid therapeutic approach to interfere with tumour invasion and metastasis.

2.2 Work package 2. Cooperation between MET, WNT and chemokines in tumour growth and invasion (Walter Birchmeier, Max Delbrück Center, Berlin, Germany)

Components of the WNT, MET and CXCR4 signaling pathways are often aberrantly active or mutated in cancer and the hypothesis underlying this work package is that cooperation between such three pathways may generate an intricate signalling network in tumour cells that promotes cancer growth and invasion. In order to test this hypothesis both human breast cancer cells were employed as well as transgenic mouse lines in which the WNT, MET and CXCR4 pathways were activated (WNT and MET) or suppressed (CXCR4).

These studies have demonstrated important and distinct roles for the WNT, MET and CXCR4 pathways during the maintenance of mammary cancer stem cells (MaCSCs) during breast cancer development. A compound WNT/MET transgenic mouse line exhibited rapid tumor formation (as early as 1 week postpartum) due to an expansion of MaCSCs (see below)

and a triple compound mutant mouse model in which active WNT/MET signalling was combined with a loss-of-function mutation in the CXCR4 chemokine receptor demonstrate a significant delay in tumour onset, hence a role for CXCR4 in this tumour model. Using specific in vitro assays, the self-renewal properties of MaCSCs (as measured in mammosphere assays) and differentiation capacity (as measured by growth in 3D-matrigel conditions) of MaCSCs were defined and found to depend on WNT and MET signalling respectively.

Furthermore, WNT-specific and MET-specific gene signatures were defined in the mouse tumour model and found to be highly predictive for human breast tumours, especially of for Basal and HER2 breast cancers. In essence, the work package generated a valuable mouse model to study the cooperation of WNT, MET and CXCR4 signaling pathways, defined a critical role for MaCSC during mammary gland tumourigenesis and provided new gene signatures predictive of certain types of breast cancer where they may guide the choice of rational approaches to therapy.

2.3. Workpackage 3. Structure of HGF/SF-MET complexes (Ermanno Gherardi, MRC Centre, Cambridge, United Kingdom)

The majority of receptor tyrosine kinases (RTK) are large, complex and multidomain proteins and the MET receptor is no exception to this rule. A key difference between the HGF/SF-MET and other RTK signalling systems, however is the structure complexity of the HGF/SF ligand: the majority of other RTK ligands (insulin, EGF, PDGF, NGF, etc) are small and simple proteins.

In contrast, HGF/SF is a complex multidomain protein, structurally related to the blood proteinase plasminogen and other proteinases of the coagulation and fibrinolytic pathways, none of which has been crystallised, so far, as intact proteins. The task of securing a crystal structure of the ternary complex HGF/SF-heparin-MET within the SFMET project, therefore, appeared hugely challenging but this task has been achieved. HGF/SF binds MET through two different regions: the N-terminal (NK1) region and the C-terminal serine-proteinase homology domain (SPHD).

The crystal structure of the HGF/SF-heparin-MET complex contained a fragment of the MET ectodomain containing the first 567 amino acids, revealed the NK1 binding site and confirmed the binding for the SPHD, which had been defined earlier in an earlier crystallographic study of the binary complex formed between the isolated SPHD and MET. Several implications emerge from the crystal structure of the HGF/SF-heparin-MET complex. First, the structure shows how MET is activated by the physiological ligand HGF/SF. Secondly, it offers new foundations for the development or optimisation of anti-MET antibodies and low molecular weight compounds with antagonistic activity.

2.4 Work package 4. Protein engineering of MET antagonists (Tom Blundell, University of Cambridge, United Kingdom)

The structural complexity of the HGF/SF protein outlined above has offered remarkable tools for structure/function analysis of HGF/SF and protein engineering of HGF/SF fragments with antagonistic activity. There are two naturally occurring splice variants of the primary HGF/SF transcript, one of which includes the N-terminal (N) and the first kringle (K1) domain (NK1); the second contains the N and the first two kringle domains (NK2).

There are important functional differences between NK1 and NK2: NK1 is a partial agonist and the basis of the partial agonistic activity of NK1 is its ability to dimerise in the presence of heparin sulphate molecules. However, mutations at the NK1 dimer interface, such as NK1_N127A, can reduce its ability to dimerise and can thus convert NK1 from a partial agonist into a receptor antagonist.

NK2 in contrast is essential a receptor antagonist and the little residual agonistic activity of NK2 can be explained by the formation of a disulphide-mediated NK2 dimer and NK2 mutants in which the cysteine residue responsible for the disulphide-mediated dimer is mutated (such as NK2_C214S) behave as bona fide receptor antagonists. Finally NK4, a large, recombinant fragment of HGF/SF containing the N domain and all 4 kringle domains of HGF/SF is a further and potent receptor antagonist currently undergoing clinical trials in cancer patients. Several structures of NK1 had been determined prior to SFMET.

As a result of the work under workpackage 3 the following new structures have been achieved: (i) the ternary complex NK1_N127A-heparin-MET, (ii) NK2_C214S, (iii) NK2_C214S-heparin complexes and, (iv) NK4. Collectively these structures have provided independent confirmation of the NK1 binding site onto MET (see WP3), have defined a structural arrangement of the N, K1 and K2 domains present in all truncated fragments of HGF/SF containing at least the first two kringle domains and have explained the basis for the antagonistic activity of the NK2_C214S and NK4 proteins.

2.5 Work package 5. Low molecular weight MET antagonists. (Ermanno Gherardi, MRC Centre, Cambridge, United Kingdom, Tom Blundell, University of Cambridge, United Kingdom, Ivano Bertini, CIRMMP, Florence, Italy)

Over the last few years progress has been made in understanding the structure of the MET kinase and the pharmaceutical industry, building on this progress has generated an extensive series of inhibitors of the MET kinase for cancer therapy. Several of these inhibitors are fairly specific (they appear to inhibit only the MET kinase and not a range of other receptor or non-receptor kinases), other display multiple specificities (MET and VEGFR is a

frequent combination) and are deemed to prove valuable for the therapy of human cancers in which two or more signalling pathways are aberrantly activated and require concurrent targeting.

There is a wide expectation that MET kinase inhibitors will constitute the first line of attack to MET in cancer but there remain reservations about the emergence of resistance to these compounds. WP5 and WP6 of SFMET, therefore, have explored alternative ways to target HGF/SF-MET signalling in cancer. Specifically, the aim of WP5 has been the generation of low molecular weight inhibitors of NK1 and MET and the aim of WP6 has been the generation of inhibitors of SHP-2, a key transducer of MET signalling and will be reported below. Both NK1 and MET display structural features that may enable targeting and inhibition via small compounds. In particular, the kringle 1 domain of HGF/SF contains a lysine-binding pocket known, from mutagenesis experiments, to play a role in MET binding.

MET also contains several pockets suitable for drug targeting although the one(s) involved in NK1 and HGF/SF binding have only been defined as a result of the work carried out under WP3 and WP4 of SFMET. In order to target NK1 and MET with small molecular weight compounds with potential antagonistic activity the structure/activity relationship of one class of compounds known to bind the lysine-binding pocket of kringle 1 has been explored and a new chemical fragment binding NK1 with good affinity from a fragment library has been isolated .

NMR experiments by I Bertini and his colleagues have confirmed the binding site of these compounds on NK1 and crystal structures of the complexes formed by NK1 and low molecular weight compound have clarified the binding mechanism at atomic level. A similar strategy has been adopted for MET-binding fragments and in all cases lead compounds have been defined that are now amenable to lead optimisation experiments via medicinal chemistry.

2.6 Work package 6. SHP-2 Inhibitors (Walter Birchmeier, Max Delbrück Center, Berlin, Germany together with Jens P von Kries, FMP, Berlin, Germany in a subcontractor capacity)

There is genetic evidence that the phosphatase SHP-2 is an essential downstream effector of MET signalling and Walter Birchmeier and his colleagues had developed a first inhibitor of SHP-2 prior to SFMET.

Under WP6 of SFMET Walter Birchmeier and his colleagues with support from Jens P von Kries's unit at FMP Berlin in a subcontractor capacity have: (i) markedly improved the potency and specificity of the original SHP-2 inhibitor via medicinal chemistry, (ii) have isolated new lead SHP-2 inhibitors from additional screens of fragment and compound (drug-like) chemical libraries, (iii) have extensively profiled both the original and new compounds on cells in culture, (iv) have characterised an in vivo model of breast cancer tumourigenesis

(the MMTV-MyPT model) for its dependency on SHP-2 and have employed this model in order to assess in vivo the anti-tumour activity of the compounds produced with striking results.

In essence, the work under WP6 has rapidly progressed both the early SHP-2 inhibitors and the new lead compounds through medicinal chemistry, has yielded proof of principle for activity in vivo and has defined safe pharmacological and toxicological profiles for the best compound in class. This work, therefore, is now ready to enter initial clinical studies.

3. Socio-economic impact

Metastastic cancer remains the main cause of cancer-related deaths and, until very recently, our knowledge of tumour invasion and metastasis was insufficient to generate inhibitors that would target cancer spreading (cell migration) as opposed to cancer growth (cell division). This situation has changed radically over the last decade with the discovery of several signalling systems with major roles in cancer invasion and metastasis and independent from roles in the growth of the primary tumour. The HGF/SF and MET system is a key example of such signalling system and has emerged in the last few years as a key target for metastatic cancer.

The work carried out as part of the SFMET project has enabled major advances in several ares. In the first instance the project has enable several breakthroughs in structural analysis of HGF/SF and MET that clarify the basis of ligand-induced MET activation and explain the mechanism of action of several receptor antagonists. These structures will also contribute to the development of new classes of inhibitors (see below).

Secondly SFMET has clarified two major functional links that govern the way in which aberrant HGF/SF and MET signalling cause cancer: (i) the link between hypoxia and MET activation has been confirmed and extended and combined inhibition of angiogenesis and MET has emerged from these studies as an important therapeutic strategy; (ii) a new link between WNT and MET appears to play a major role at least in breast cancer, and possibly in other tumours, via cancer stem cells and this discovery too will act as the basis for new therapeutic strategies.

Thirdly, SFMET has demonstrated that, in addition to inhibition of the MET kinase, there are other strategies for achieving functional blockade via low molecular weight compounds and these include inhibitors of NK1, MET and SHP-2 and, although the NK1 and MET inhibitors will require further medicinal chemistry, the SHP-2 inhibitors have been taken by the project on the threshold of clinical studies.

In summary, the SFMET project has produced major advances in structural biology, cancer biology and drug discovery that have extended our understanding of cancer and offered the foundation for practical medical progress.

4. List of Beneficiaries:

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