Institution: University of Dundee

Unit of Assessment: 5: Biological Sciences

Title of case study: Impact of research on AMP-activated protein kinase on the instigation of clinical trials testing the effect of the diabetes drug metformin on reducing cancer incidence and on the development of new therapeutics for diabetes and metabolic syndrome.

1. Summary of the impact (indicative maximum 100 words)

In 2003, researchers at the University of Dundee identified the tumour suppressor LKB1 as a critical upstream activator of AMP-activated protein kinase (AMPK), which provided the first link between AMPK and cancer. Metformin, the front-line therapy for type-2 diabetes, was already known to exert its beneficial effects through AMPK. An interdisciplinary collaboration at the University examined the link between metformin and cancer, and reported in 2005 that diabetics taking metformin had a reduced incidence of cancer. The impact has been clinical trials worldwide testing the benefit of metformin for cancer treatment, and development of therapeutics by pharmaceutical companies targeting this pathway.

2. Underpinning research (indicative maximum 500 words)

In 1987, **Prof. Grahame Hardie FRS** (Professor of Cellular Signalling at the College of Life Sciences) first defined AMP-activated protein kinase (AMPK) as a protein kinase that inactivated acetyl-CoA carboxylase (ACC) and HMG-CoA reductase. In a series of seminal papers published between 1988 and 1996, he elucidated the regulation of AMPK by adenine nucleotides and phosphorylation, identified the critical phosphorylation sites on ACC and on AMPK (still universally used as biomarkers for AMPK activation today), developed the peptide substrate used for its assay (still in wide use), and identified AMPK as a heterotrimeric complex with a catalytic α subunit and regulatory β and γ subunits. AMPK is now known to be a key player in regulating energy balance at both the cellular and whole-body levels, placing it at centre stage in studies of obesity, metabolism and diabetes (1).

In 1996, Prof. Hardie showed that another protein kinase was required to activate AMPK by phosphorylating threonine-172 within the kinase domain on the α subunit (2). In 2003, by mining the yeast genome, he identified three protein kinases acting upstream of the yeast orthologue of AMPK (3). The closest mammalian relative to these was LKB1, previously identified as a tumour suppressor responsible for an inherited cancer syndrome (Peutz-Jeghers syndrome). With **Prof. Dario Alessi FRS** (Professor of Signal Transduction at the College of Life Sciences), Prof. Hardie made the crucial discovery that LKB1 was the elusive upstream kinase required to phosphorylate threonine-172 on AMPK (4). It has been subsequently found that LKB1 is mutated in many spontaneous cancers, including 30% of non-small cell lung cancers and 20% of cervical cancers; this may promote tumourigenesis by removing the restraining influence the LKB1-AMPK pathway normally has on cell growth and proliferation.

The crucial link between LKB1 and AMPK made by Hardie and Alessi stimulated an interdisciplinary collaboration with **Prof. Andrew Morris FRSE FMedSci** (Professor of Diabetic Medicine and Dean of Medicine at the University of Dundee). The finding that LKB1 activated AMPK was intriguing given that AMPK was known to be activated in cells by the widely used diabetic drug, metformin. This gave rise to the hypothesis that AMPK activation by metformin could decrease the incidence of cancer. Professor Morris, an expert in the use of informatics to study the epidemiological and molecular aetiological basis of diabetes and its complications, tested this hypothesis using unique record linkage databases developed in Tayside, Scotland: a diabetes clinical information system (DARTS) and a database of dispensed prescriptions (MEMO). The results of the epidemiological survey, published in the British Medical Journal, revealed that diabetics taking metformin were 30% less likely to develop any form of cancer than those on other medications (5). In follow up experimental work by Prof. Alessi, metformin and other AMPK activators were also found to delay the onset of tumourigenesis in cancer-prone mice (6). These studies sparked huge interest worldwide both in the use of metformin for cancer prevention and treatment, and in new drug development programmes to find novel activators of AMPK.

3. References to the research (indicative maximum of six references)

1. **Hardie, D.G.,** Carling, D. and Carlson, M. (1998) The AMP-activated/SNF1 protein kinase subfamily: metabolic sensors of the eukaryotic cell? Ann. Rev. Biochem. *67*, 821-855 (doi: 10.1146/annurev.biochem.67.1.821) (Citations 956, Scopus Nov 2013)

2. Hawley, S.A., Davison, M., Woods, A., Davies, S.P. Beri, R.K., Carling, D. and **Hardie, D.G.** (1996) Characterization of the AMP-activated protein kinase kinase from rat liver, and identification of threonine-172 as the major site at which it phosphorylates and actvates AMP-activated protein kinase. J. Biol. Chem. *271*, 27879-27887. (doi:10.1074/jbc.271.44.27879) (Citations 504, Scopus Nov 2013)

3. Sutherland, C.M., Hawley, S.A., McCartney, R.R., Leech, A., Stark, M.J., Schmidt, M.C. and **Hardie, D.G.** (2003) Elm1p is one of three upstream kinases for the Saccharomyces cerevisiae SNF1 complex. Curr. Biol. *13*, 1299-1305. (doi:10.1016/S0960-9822(03)00459-7) (Citations 146, Scopus Nov 2013)

4. Hawley, S.A., Boudeau, J., Reid, J.L., Mustard, K.J., Udd, L., Makela, T.P. **Alessi, D.R.**, and **Hardie, D.G.** (2003) Complexes between the LKB1 tumour suppressor, STRADα/β and MO25α/β are upstream kinases in the AMP-activated protein kinase cascade. J. Biol. *2*, 28. (doi:10.1186/1475-4924-2-28) (Citations 792, Scopus Nov 2013).

5. Evans, J.M., Donnelly, L.A., Emslie-Smith, A.M., **Alessi, D.R** and **Morris, A.D**. (2005) Metformin and reduced risk of cancer in diabetic patients. BMJ. *330*, 1304-1305. (doi:10.1136/bmj.38415.708634.F7) (Citations 577, Scopus Nov 2013).

6. Huang, X., Wullschleger, S., Shpiro, N., McGuire, V.A., Sakamoto, K., Woods, Y.L., McBurnie, W., Fleming, S. and **Alessi. D.R.** (2008) Important role of the LKB1-AMPK pathway in suppressing tumorigenesis in PTEN-deficient mice. Biochem. J. *412*, 211-221. (doi:10.1042/BJ20080557) (Citations 142, Scopus Nov 2013).

4. Details of the impact (indicative maximum 750 words)

Beneficiaries (the benefit/impact):

(a) Pharmaceutical companies (the identification of AMPK agonists as cancer prophylactic and potential therapeutic agents, as well as for diabetes)
(b) Diabetes patients (patients in Phase II clinical trials who have benefited from Imeglimin^{TM})
(c) Cancer patients (patients in Phase II clinical trials who have benefited from metformin or other AMPK agonists in their therapy)

Impacts:

Drug Development campaigns by Pharma for novel AMPK activators

Research by the University of Dundee not only identified the components and regulation of the AMPK signalling cascade, but also defined AMPK as a key energy sensor with a central role in metabolism, diabetes and, more recently, cancer. The impact of AMPK research at Dundee can be evidenced by the publishing of 57 patents from 22 different organisations, describing small molecule activators of AMPK. This, in turn, has led to the initiation of several major drug discovery campaigns and clinical trials, including:

Poxel, a spin-off from Merck-Serono in 2009, has developed both a new indirect activator of AMPK called Imeglimin^{TM}, as well as a direct activator of AMPK that is in pre-clinical development (1). Imeglimin^{TM} is currently undergoing Phase II clinical trials to test the benefit of additive effects of Imeglimin^{TM} with metformin. In 2012, Phase IIa clinical trials showed that Imeglimin^{TM} displays a superior benefit : risk profile compared with metformin in type 2 diabetes patients (2), and Phase II results recently released by Poxel suggest that Imeglimin^{TM} showed increasing effectiveness as an add-on therapy to Sitagliptin in patients inadequately controlled by Sitagliptin monotherapy.

Mercury Pharmaceuticals Inc has developed new compounds to activate AMPK for the treatment of prostate cancer (3). The company is evaluating these compounds in an established animal model of human prostate cancer and initial results are very promising. In addition to prostate cancer, genetic markers in 30%-50% of non-small cell lung carcinomas, 40% of colon cancers and over 50% of malignant melanomas suggest that AMPK activators developed by Mercury Pharmaceuticals Inc will have efficacy in these indications. In 2010, the company signed an exclusive agreement with Debiopharm Group for the development and commercialisation of a small molecule activator of AMPK (Debio 0930) for treatment of type 2 diabetes (4).

Betagenon, performed a drug discovery programme to develop novel direct AMPK agonists (5). In 2008, the pharmaceutical company Antisoma announced that they had licensed rights to develop and commercialise Betagenon's AMPK activators in cancer indications.

Clinical trials of metformin for cancer treatment and prophylaxis

The identification by the Dundee team that the tumour suppressor LKB1 is the upstream kinase that activates AMPK, together with the finding that metformin decreases cancer incidence in diabetic patients, has had a major impact on the cancer field, especially as metformin is orally available, has no long-term safety issues and is available as a generic drug. This has driven 52 case-controlled clinical trials worldwide (many between 2008-2013) with an enrollment of over 7,000 people in 10 different countries. As an example, these trials include one started in 2010 as the first large-scale international clinical trial testing the effects of metformin administered for 5 years on both recurrence and survival in early-stage breast cancer (6). These kinds of trials will establish whether the stratification of patients with AMPK-inactivation in their cancers will benefit from AMPK-activating drugs and also assess whether existing chemotherapy and radiotherapy regimens may be enhanced by combining them with treatments that modulate AMPK. Thus far, two pilot studies, in 2011 and 2012, have demonstrated a positive effect of metformin in women with newly diagnosed breast cancer awaiting surgery (7-8): short-term preoperative metformin was well-tolerated and resulted in clinical, biomarker and cellular changes consistent with beneficial anti-cancer effects.

As of 2013, there are 13 Phase I, 37 Phase II and 2 Phase III trials underway examining the effect of metformin in a variety of cancers such as pancreatic, prostate, colorectal, and breast cancer (see http://clinicaltrials.gov/) and as of October 2013, metformin appears promising as a preventive agent for ovarian and hepatic cancers. Whilst anecdotal, Lewis Cantley, Director of the Beth Isreal Cancer Center, Harvard Medical School, said of the drug: "Metformin may have already saved more people from cancer deaths than any drug in history".

5. Sources to corroborate the impact (indicative maximum of 10 references)

1. Further information can be found on the Poxel website http://www.poxel.com/pipeline
2. Pirags V., Lebovitz H. and Fouqueray P. (2012). Imeglimin, a novel glimin oral antidiabetic, exhibits a good efficacy and safety profile in type 2 diabetic patients. Diabetes Obes Metab. *14*, 852-858 (doi: 10.1111/j.1463-1326.2012.01611.x).
3. Further information can be found on the Mercury Pharmaceuticals Inc website http://www.mtipharm.com/
4. Details of the Debiopharm - Mercury agreement, and the Debio 0930 compound can be found at http://www.mtipharm.com/news/pr20090408.htm.
5. Further information can be found on the Betagenon website http://www.betagenon.com/
6. A Phase III Randomized Trial of Metformin Versus Placebo on Recurrence and Survival in Early Stage Breast Cancer Principal Investigator: Dr Pamela J. Goodwin, Mount Sinai Hospital, New York. **ClinicalTrials.gov Identifier:** NCT01101438

http://clinicaltrials.gov/ct2/show/NCT01101438

7. Niraula, S., Dowling, R.J., Ennis, M., Chang, M.C., Done, S.J., Hood, N., Escallon, J., Leong, W.L., MCready, D.R., Reedijk, M., Stambolic, V., and Goodwin, P.J. (2012) Metformin in early breast cancer: a prospective window of opportunity neoadjuvant study. Breast. Cancer. Res. Treat. *135*, 821-830. (doi: 10.1007/s10549-012-2223-1)

8. Hadad, S., Iwamoto, T., Jordan, L., Purdie, C., Bray, S., Baker, L., Jellema, G., Deharo, S., Hardie, D.G., Pusztai, L., Moulder-Thompson, S., Dewar, J.A., and Thompson, A.M. (2011) Evidence for biological effects of metformin in operable breast cancer: a pre-operative, window-of-opportunity, randomized trial. Breast. Cancer. Res. Treat. *128*, 783–794 (doi: 10.1007/s10549-011-1612-1)