Int. J. Dev. Biol. 48: 457-462 (2004)

From here to there; a life based on migration

An interview with Isaiah J. Fidler

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As part of the technological advances of our age, it proved possible to conduct this interview across "the ether" using the medium of e-mail. Not the most satisfactory medium for me and not just because I failed to add to my air miles by foregoing a trip to Houston. It was disappointing for me because, as a former pupil of Josh's, I was well aware of the warm and generous hospitality I could have expected from him and his wife Margaret had I visited their home. However of greater import for the flavour of this review is the fact that by not going to Houston I am forced back on fairly distant memories that could have done with being refreshed. Josh is one of the great teachers in modern tumour biology and his teaching skills are indivisible from his personality (his larger-than-life personality). Just as people say you have to see great rock bands, like the Rolling Stones, on stage in order to appreciate how good they are, so you need to have seen Josh Fidler lecturing in the flesh in order to really appreciate what an impact he has had on the field of metastasis research. To have "bathed" in that charisma again after a long absence from personal interaction would have helped gel some of the points I would like to get across in this interview. Hopefully though I still will be able to convey the excitement of working under Josh's direction in the late 70's and early 80's; it was a time when Josh made a number of seminal observations and drove the field forward almost by his sheer will-power. Before we leave the analogy drawn to rock musicians though, let me encourage any reader that, should Fidler be playing at a venue near you in the near future, a trip to a live-lecture will always be an event on a par with anything the world of rock-and-roll has to offer!

Tell me about your early days and how you decided to pursue a career in science?

I was born in Israel and, having completed my military service, with adulthood came a desire to build on my love of animals and turn it into a career by becoming a veterinarian. Accordingly I came to the USA for the first time, enrolling in Oklahoma State University from which I graduated as a DVM [ed. Doctor in Veterinary Medicine] in 1963. I returned to Jerusalem and established my own veterinary practice, but like many colleagues I found routine practice unrewarding. Parenthetically it is interesting that both you Ian and George Poste, my good friend with whom I did such exciting work in the 1970s-1980s along with Garth Nicolson, also took this route of migrating out of veterinary practice and into metastasis research; perhaps there is something in the 'veterinary water' which gives us an interest in the topic. Whatever, I decided that my intellectual aspirations were not being met by my routine veterinary work in Jerusalem and I returned to the USA in 1966 to the School of Veterinary Medicine, University of Pennsylvania, Philadelphia. Here I was fortunate enough to come under the influence of Robert Brodey, the pre-eminent veterinary oncologist of his time, who opened my eyes to the intellectual fascination of cancer. I decided that oncology, and surgical oncology in particu-

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0214-6282/2004/$25.00

Printed in Spain
www.ijdb.ehu.es

lar, was to be "my field" and my work with Bob Brodey led to my first scientific papers which generally were on mammary tumours in dogs (e.g. Brodey *et al.,* 1966; Brodey and Fidler 1966; Fidler *et al.,* 1967). We were even fortunate enough, as veterinarians, to have a paper on the treatment of a dog accepted by that bastion of the medical profession, "The Lancet" (Old *et al.,* 1967).

Notwithstanding how good I became as a surgical oncologist I still lost many of my patients and they almost always succumbed to metastatic spread rather than to the consequences of the primary tumour. I determined therefore to learn more about this process and the driving rationale behind this wish was my view that if you didn't understand how something was broken you were unlikely to be able to mend it! I wanted to know how cancer cells managed to spread around the body. Having discussed the matter with Robert Marshak, my Chairman, I decided to apply to Graduate School at University of Pennsylvania to undertake a PhD in mechanisms of cancer metastasis under the direction of Irving Zeidman, then Professor of Pathology. This I did in 1968 and the die was cast; from thence forward I was to be a student of the most important behavioural characteristic of malignant tumours.

Were there any substantial differences between biological laboratories then and how they are now?

Well it was a wonderful time to be embarking in this nascent scientific field (metastasis was very much the poor relation in terms of the interest it generated in the scientific community) and in that respect I felt the same excitement that many young graduates feel today who are embarking on careers in rapidly-expanding areas. However one of the things which has struck me on looking back to this period is the allowance of time which I was so fortunate to be granted. There wasn't the unthinking type of frenetic activity so characteristic of many modern laboratories. Nowadays the need to generate data is pre-eminent as a pressure on young scientists. Instead, then we started the day with a coffee and discussion period at which we would pore over recent results, both ours and those recently published by other groups, and try to formulate questions which we could address in the laboratory. Often these questions were of a rather general nature rather than the more limited and restricted nature so beloved of most of us in this reductionist age. My early experience gave me the luxury of trying to sort the big from the peripheral questions. Perhaps we now are seeing a return to a more holistic approach to cancer biology but certainly even in those days we were well aware of the interactions between a responding host and the transformed cell and how this dynamic reciprocity could affect the outcome of metastatic spread. Once I became a mentor myself I often counselled my students to take time to think; not to rush in and do an experiment simply because the reagents were available. Rather they should try and plan out exactly what it is that they are trying to address and to devise the best experiments they can which are directed at answering this central point. While the literature now is huge, defying easy digestion or discussion, the core central questions remain but I am a little doubtful that the somewhat "laid-back" approach of the Zeidman lab is replicated in too many places nowadays.

Anyway, regardless of whether it was a more enjoyable time 30 years ago, it certainly was the consideration of the dynamic interaction, between host and disseminating cancer cell, which led to my thesis project. Using the VX-2 rabbit carcinoma, Irv Zeidman had shown that an incredibly large number of viable tumour cells, at least one million, had to be injected directly into the circulation in order to give rise to a very small number of experimental metastases in either the liver or the lungs. Why? What was happening to the cells after injection? Where did they go? Were they alive or were they dead? In part the difficulty seemed to me that we had no effective way of monitoring the cells once they had entered the "black box" of the entire animal. In these days of GFP-tagging of tumour cells, this would seem to be a highly tractable problem. However in those days it was a little more problematic and seemed to best be approached by using radioisotopes to "tag" the cells. I hit upon the concept of using [^{125}I] iododeoxyuridine to label the DNA of tumour cells, rather than the chromium-51 labelled tumour cells being used by Bernard and Edwin Fisher in their very comparable studies ongoing in Pittsburgh. This was because this technique allowed me to follow the fate of living cells; a situation which was not necessarily the case with ^{51}Cr labelling. This, coupled with the use of the B16 melanoma which gave rise to pigmented lung lesions which were easily visible (an original suggestion to me by my fellow postdoctoral fellow, John Kreider), meant I was able to document the fate of i.v. injected tumour cells and show fairly easily that secondary deposits resulted from the survival of only a very few, or a select population, of these introduced cells (Fidler, 1970). The fact that most cells die in the circulation, and die fairly rapidly, left me with a major question. Do grossly evident secondary deposits result from the preferential survival of a metastatic subpopulation or do they represent a random occurrence? To answer this question I made good use of the pigmented nature of the B16 melanoma which allowed me to harvest lung tumour nodules with relative ease. I injected C57BL6 mice intravenously with B16 cells, allowed the animals time to develop visible lung lesions, killed the mice and removed the lungs, harvested these pigmented lesions and re-established the tumour cells in culture. I then injected them back into new groups of recipient mice and I repeated this cycle several times. Eventually I found that, for the input of the same number of tumour cells, the selected metastatic tumour cells (now labelled B16F10) gave significantly more lung nodules than their parental counterparts (B16F1). My conclusion from these experiments was that metastasis was a selective event and this was the message I put forward in my report which was published (in the face of some scepticism on my colleagues' part as to the choice of journal I might say) in "Nature" (Fidler, 1973).

As an aside I should mention here that both my paper in "JNCI" and this paper in "Nature" did not bear the name of Irving Zeidman in whose Department the work was done. It indicates the generosity of Irv in letting me establish myself as an independent worker in the field of metastasis research without claiming any recognition for himself. It is tempting to think that the pressure to publish all the time, as an absolute requirement to garner research funds, is responsible for the way that so many senior figures now append their name to any and every paper emerging from their Department, whatever their intellectual contribution. Perhaps Irv was not under these same pecuniary pressures but the more gentlemanly ways of Penn. in the early 70's are to be envied!

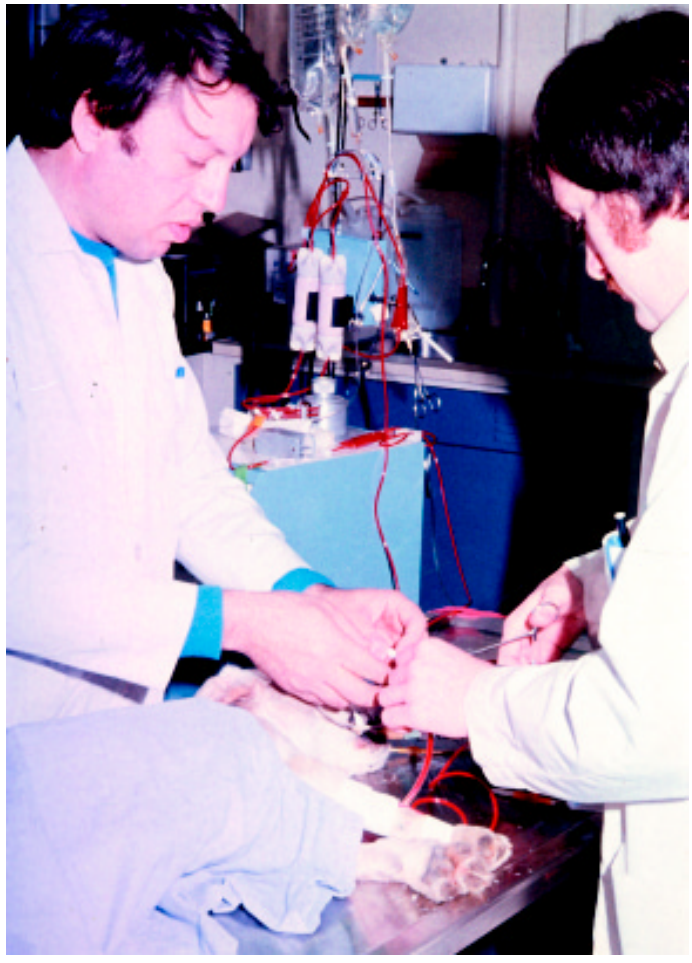
Did anything else of significance happen around this time?

Well it was in 1973 that I was naturalized as a US citizen. Of course you can take an Israeli out of Israel but you cannot take

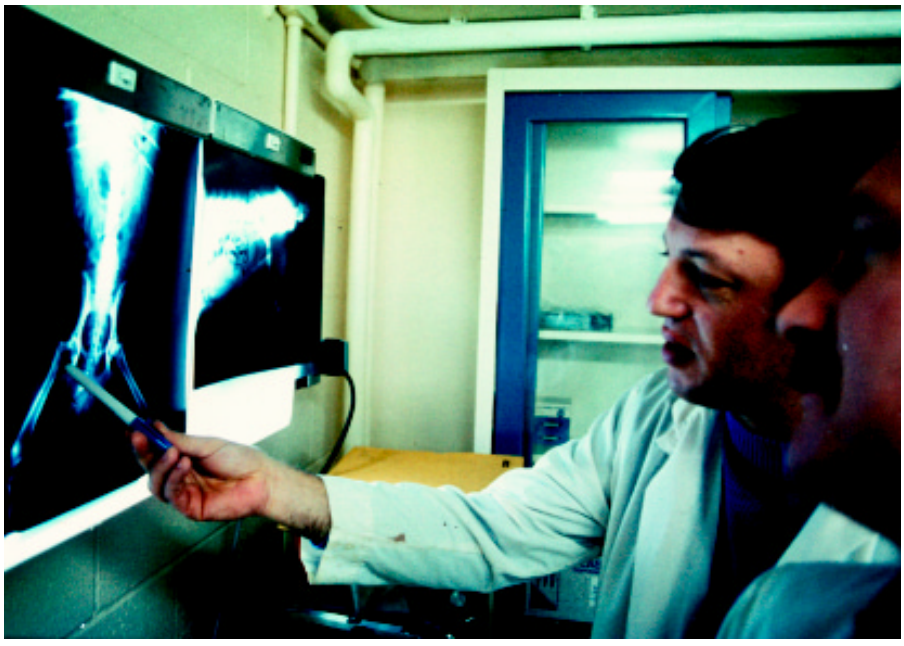
Israel out of an Israeli and, even with my change of status, I still hold huge affection for my homeland. Having said that, the USA has been the home in which I have conducted my entire scientific career and, having been fortunate enough to have enjoyed the fruits of this support, it seems only reasonable that I should be a full citizen of this wonderful country.

What happened after that in terms of personal and scientific evolution?

Well of course around that time there were other major changes in my life. Firstly I met and married my wife Margaret Kripke and secondly we were both recruited as independent heads of laboratories in the newly established Cancer Biology Program at the NCI Frederick Cancer Research Facility. This was situated in Building 539 in the old biological warfare site of Fort Detrick in Frederick, Maryland. It was a time of great scientific excitement and I am indebted to my friend, Michael Hanna Jr., for recruiting both Margaret and me to this Program. Perhaps the best aspect of this move was that our personal and scientific destinies became so intermixed while the juxtaposition of our respective laboratories allowed us to work together as full and frequent collaborators. The happiness of my time working in Frederick was increased further by the fact that, as a Lab. Head, I was able to recruit and work with a number of young scientists including Doug Gersten, Nabil Hanna, Avraham Raz, Jim Talmadge and Raffaella Giavazzi, as well as yourself Ian, to name but some. Indeed, it is a source of considerable pleasure to me that many of these people continue to work on various aspects of research into the problem of cancer metastasis. I have always enjoyed teaching and the success of many of my pupils has given me considerable satisfaction; satisfaction which has continued in my present location in Houston where I have been equally fortunate in overseeing the work of a similarly talented band of young scientists and clinicians. However, to return to the chronology of this scientific journey, of major note during this period was that it was here in Frederick that I conducted the work that led to what I consider to be my seminal paper of all the many that I have published. Of major import here, and again a source of great satisfaction, was that this work represented a wonderful collaboration between Margaret and myself. It arose from comments that Margaret made regarding the work that I already had published on the selective nature of the metastatic process. An obvious alternative inference from the results on the derivation of the B16F10 from the B16F1 line would be that there had been an adaptation process which had allowed the F10 cells to give rise to a greater number of metastases. This could have been the factor which caused the increase in lung tumour nodule formation rather than that the process of selection had pulled out a pre-existent sub-population. Margaret, who had had training in both Immunology and Microbiology, suggested that this problem could be nailed by modifying the Luria and Delbruck fluctuation assay which was well known in microbiology for its formal demonstration that virus resistant bacteria pre-existed within a parental population. Margaret and I adapted this classical assay to look at the development of experimental metastasis. The way we did this was to clone the B16 melanoma and show that the various clones, each of which was derived from a single cell, differed dramatically and significantly in their ability to form pulmonary metastases after i.v. injection into cohorts of syngeneic mice. Sub-cloning experiments showed that the observed diversity bred true i.e. that it was not induced by the cloning procedure *per se*. Control aliquots of the B16 parental population, which simply had been subcultured for the same period of time, showed a very narrow range of ability to form experimental tumour nodules in cohorts of other mice. The paper, which was published in "Science" (Fidler and Kripke, 1977), showed that the metastatic heterogeneity found in the B16 melanoma pre-existed. In other words our ideas that metastasis was a selective process which allowed for the emergence of the metastatic variants, were shown to be correct. Of course, even in 1977, the B16 melanoma had been in culture for a prolonged period of time and there was a formal possibility that it was this length of culture time which had induced this phenotype. It was of considerable interest then that we were able to show the same degree of metastatic heterogeneity in a recently established murine fibrosarcoma (Kripke *et al.,* 1978) and a melanoma line, K-1735, that grew in C_3H mice (Fidler *et al.,* 1981). In line with my efforts to "keep things in the family" during this period of my work both the UV-2237 fibrosarcoma and the K-1735 melanoma had arisen from the UV-based carcinogen studies that Margaret had been conducting at Frederick. No such connection was apparent in our subsequent demonstration that similar metastatic heterogeneity was to be found in human melanoma whose disseminative proclivity could be assessed in athymic nude mice (Kozlowski *et al.,* 1984) but this last



A young Josh Fidler *(left) and an even younger and more hirsute Ian Hart conducting leukophoresis on a dog (1977).*



Josh Fidler and Ian Hart *(with a '70's moustache) examining the radiograph of a dog with osteosarcoma (1977).*

report did set the seal on a hypothesis which has been the "central dogma" of metastasis research for twenty to thirty years. What we had shown was that metastatic subpopulations pre-existed within the primary tumour mass. Given that a metastatic deposit can only arise when tumour cells have detached, invaded, survived in the circulation, attached, extravasated, proliferated and induced a neovascular response while evading host defence mechanisms (Fidler, 1990), this inability of most tumour cells to complete the process does not seem surprising. However because metastases are largely clonal in origin, as Jim Talmadge and I showed in the early 1980's (Talmadge *et al.,* 1982; Fidler and Talmadge 1986), it seems apparent that the successful metastatic cell must have a full set of characteristics which enable it to complete each of these steps. The past twenty years have largely been spent identifying these characteristics and determining how they differ between metastatic and non-metastatic variants of the same tumour type.

This view has changed somewhat hasn't it with the recent development of microarray analysis?

Well yes. The best known experiments to which you refer are, for example, those of Ramaswamy *et al.,* (2003) in which they have looked at gene array analysis of human primary tumours and the metastases of multiple tumour types. What these workers found was that there were gene expression signatures which distinguished primary from metastatic adenocarcinomas. This, of course, is in line with our views on how metastases develop. Rather more contentiously, from our point of view, they also found that a subset of primary tumours resembled metastatic tumours with regard to a specific gene expression signature. The inference of these results was that the metastatic potential of human tumours is encoded in the bulk of a primary tumour and that solid tumours carrying this gene expression signature will most likely be associated with metastasis and a poor clinical outcome (Ramaswamy *et al.,* 2003). The authors have used these results to argue that "the notion that metastasis arises from rare cells within the primary tumours is probably wrong"; a direct questioning of the "central dogma" established by me. However. I myself do not see this stark contradiction between our two sets of data. We already know from *in situ* hybridisation experiments, for example, that many genes are differentially expressed within the primary tumour and that expression or repression of these genes may give rise to a high likelihood that the tumour eventually will give rise to metastasis. What I believe that Ramaswamy *et al.,* have done, using a genome-wide analysis, is to provide data which are consistent with those which have been derived from *in situ* hybridisation. That is that the expression of specific genes is a pre-requisite for metastatic proclivity. Whether these traits are present early or late in tumour evolution does not alter the fact that, like the decathlon athlete, tumour cells capable of forming secondary deposits must be able to complete each of the steps in the metastatic sequence. There is a considerable amount of evidence showing that progression from benign to malignant in the major solid cancers is a consequence of the acquisition of a series of genetic and epigenetic alterations which give rise to the phenotype characteristics of malignancy. These alterations accumulate at different rates in different tumours and, of course, form the basis of the pathologists' assignation of clinical cases to various tumour stages. The early breast cancers and the stage I and II (early) lung adenocarcinomas studied by Ramaswamy *et al.,* generally expressed the non-metastatic gene expression pattern with very few expressing the metastatic pattern. This probably does reflect the fact that some of the primary tumours indeed have generated specific cells with full metastatic capacities. Indeed the study represents an important step in our attempts to predict tumour behaviour at an early stage. Nonetheless I do not think this particular study truly addresses the question of the prevalence of fully metastatic cells in primary tumours. A major advantage of these types of studies is that it also gives us the opportunity to identify genes which were previously unknown or not expected to be involved in the metastatic process and we might identify new 'players' via this technique. However, I must say that all concepts in biological science are put forward and then modified in the light of new data which may be derived from new techniques. I see no problem with my views being questioned as a consequence of new findings. Should the results that Ramaswamy *et al.,* (2003) present hold up across a wide spectrum of tumours and indicate that metastatic signatures already pre-exist within primary tumours then I would not feel that the paper that Margaret and I wrote in 1977 had been rendered redundant in any way. As a consequence of our results and our report in "Science", numerous attempts have been made to identify the genes responsible for the differences in metastatic behaviour. These studies have relied on variants derived from within the same parental population thus obviating the confounding difficulties of comparing "apples with oranges" and from such studies has come elucidation of a role of a number of important

genes in cancer spread. I can name for example E-cadherin, the matrix metalloproteinases, the integrins, the hypoxia-induced genes all as examples of genes which have been shown to contribute to the metastatic process, and all of which I feel would have been longer in being associated with tumour dissemination had our concept of metastatic heterogeneity not previously been brought forward. The purpose of scientific papers is to stimulate further research and the fact that our "Science" paper has been cited thousands of times is ample evidence of the impact it has had on the field.

What happened next and where did you go after Fredrick?

Well in 1983 I was recruited to my present position as RE "Bob" Smith Distinguished Chair in Cell Biology at the University of Texas, MD Anderson Cancer Centre, Houston. Margaret, as befits a scientist of her stature, was recruited at the same time to be Chair of the Department of Immunology. Both Margaret and I were sad to be leaving Frederick but the opportunities that have existed in Houston, and the cadre of workers whom I have recruited since arriving in Texas, has fully justified our decision to come so far into the deep South.

Since being in Houston, a major contribution to the practical aspects of utilisation of cancer models has been your work on orthotopic transplantation. Would you agree?

Yes I would. It always has struck me that the desire to use models of human tumours in which metastatic behaviour has been assessed simply by placing the tumours in the subcutaneous site is rather naive. It has ignored the important aspects of local host tumour interactions; considerations which necessitate the need to implant the tumours in their sites of original development. For example colorectal cancers should be implanted in the colon, prostate cancer should be implanted in the prostate etc, etc. Once one does that one finds there are significance differences between the behaviour of tumours implanted in different anatomical locations of the recipient animal hosts. To my mind these possibilities may well explain our past difficulties in obtaining chemotherapeutic agents which are effective against disseminated disease.

Was this an original idea of yours?

Well as one of my old students, Ian I hope you will remember that one of my best aphorisms was that which said "If you think you have had a good idea, look in the library"! My good idea certainly was supplemented by my looking in the library. Thus I was well aware of the paper by Tan *et al.,* (1977), published in the "Journal of the National Cancer Institute", which showed that murine colon adenocarcinoma cells when transplanted in the sub-mucosa of the caecum grew and metastasized far better than when the tumour was implanted in the sub-cutis. These authors suggested that orthotopic transplantation as a model system can provide an important way for assessing metastatic tumour spread. While their initial report was in regard to the role of the local immune response I felt that their results had far more widespread ramifications. While they followed with work on pancreatic cancer and showed that the human pancreatic cancer line AsPC-1 implanted orthotopically in to nude mice (Tan *et al.,* 1985) metatasized much better than it did from s.c. site they did not pursue this work very much. I on the other hand have spent a lot of time investigating the feasibility of implanting human tumours into athymic mice and showing that significant differences in behaviour may be obtained dependent on the site of tumour implantation (e.g. Giavazzi *et al.,* 1986 a,b, Naito *et al.,* 1986, 1987 and Stephenson *et al.,* 1992). To me this makes biological sense and using such systems we have been able to complete a number of pre-clinical evaluations of drugs and biological treatments which have then been taken into the clinic by a number of my clinical collaborators. Indeed it is a matter of much regret to me that, because of the constraints of space, this Interview is going to end with my work only up to the early 1990's. Since that time I have been devoting more and more energy toward trying to get novel therapeutic approaches to metastatic cancer into the clinic. I realize that this area does not come within the remit of those areas you were asked to cover by the Guest Editors and thus must be left on the "cutting room floor" but I'd be pleased to expand, and expound, on these efforts in thirty years time if we're asked to do this again! I have always said that a model is only as good as the question to be asked and addressed by that model. Clearly with metastasis the most important question to be asked is "Can we treat tumours in disseminated sites?" and for this we need a model with true clinical relevance. My belief is that the orthotopic models provide us with more clinically relevant model systems; a huge boon for future therapeutic screens and an approach I have been using in my work over the last decade.

The leaving party for Josh and his group *on their departing Frederick in 1983. From left to right: Ian Hart, Bobbie Jones (Josh's PA) and Josh Fidler.*

You have been a very prolific speaker giving talks and lectures at numerous international conferences. Is this an aspect of your work which you have enjoyed and has this helped to get your 'message' across?

I have always said that I am a frustrated stand-up comic. Had I not been a scientist I would have enjoyed being a comedian and those who know me well know that my talks frequently are interspersed with anecdotes that reflect both my view of science and my view of life. This has certainly helped to get my message across because a talk with humour is bound to be more memorable than one without. I might add that the use of humour has had more tangible benefits for me too. Margaret has a most infectious giggle and it was the sound of her response to one of my jokes at a Gordon Conference presentation which first drew her to my attention. Imagine if I had had a deletion in the humour gene just how different my personal and scientific life could have been! Overall then I enjoy presenting science, which I believe is an important aspect of a scientist's development and one which we often ignore in our training of young people. If you don't believe your own work is exciting and fascinating how are you going to convince an audience that your work is interesting? Again I am proud of the fact that many of my protégées have turned out to be good presenters; a facet I attribute to my insistence on their presenting their talks to me prior to delivering them to a wider audience.

Where do you see work on metastasis going?

Well, as we have discussed already, the utilisation of microarrays has allowed for significant advances in analyzing the basis of metastatic development. Obviously this type of study and analysis will continue in the foreseeable future, and will be supplemented with sophisticated proteomics analysis. However, in my opinion, what it may do best and where it may better be utilised is that such work may enable the identification of the subset of tumours which, by normal pathologic criteria, we would designate as early stage but which already have released a few metastatic cells. Thus the new approaches taken could be important in detecting and predicting the behaviour of tumours at an early stage and such information could allow the stratification of patients into different treatment arms. I too believe that we now know most of the molecular players in the metastatic process and that few new candidates are likely to be discovered in the future. Rather what we need to do is establish how we can identify patients in whom changes in the expression of these genes determines prognostic outcome and to apply the results of such analyses to the development of novel therapies.

What aspect of your career would you change if you had the time over again?

Well it sounds a little smug to say so but I don't think that I would change anything. I have been very fortunate in having developed a concept which has stood for almost 30 years and which has driven forward an awful lot of work on a broad front. This has been in the area of tumour biology which I, as a trained surgical oncologist, have considered to be the most important aspect of oncology. When I first started you could have gathered together all the investigators on metastasis into a small tutorial room. Now we have a whole scientific society dedicated to investigation of this aspect of tumour behaviour and consideration of these points is now a major part of any major cancer meeting. I like to think that at least some of this increase in interest is attributable to my influence. Moreover I have been fortunate to have trained many investigators and have always enjoyed my interactions with these enquiring minds. What I have been fortunate enough to teach them has, I hope, stood them in good stead throughout their scientific careers. I have enjoyed working with a wide range of collaborators who have been good and true friends, but perhaps most importantly of all, I have been fortunate in having as my closest friend and colleague, my wife, Margaret. I could not have asked more of a career.

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