

## NEWS

# Conversations with pioneers in the bone field: T. John Martin

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International Bone and Mineral Society, Chicago, IL, USA.

IBMS BoneKEy 10, Article number: 339 (2013) | doi:10.1038/bonekey.2013.73; published online 24 April 2013

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What's in a name? For a molecule like PTHrP, quite a lot

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*'Many of the previously puzzling aspects surrounding the HHM [humoral hypercalcemia of malignancy] syndrome are explained by the existence of a separate gene encoding a protein related to PTH, which is sufficiently different to be undetected by most PTH antisera.'*

So concluded Jack Martin and colleagues in PNAS in July 1987, in an article reporting the discovery of a new molecule they called parathyroid hormone-related protein (PTHrP). Among many enormously influential findings in the bone field, the discovery of PTHrP is a highlight of Professor Martin's highly distinguished career dedicated to investigating calcium-regulating hormones, bone cell biology, cancer and bone, and much more. Professor Martin recently sat down with BoneKEy for the inaugural interview for Conversations With Pioneers in the Bone Field, a new series of articles to be published in BoneKEy. Professor Martin discusses the discovery of PTHrP, subsequent research on the protein's function—both in bone and non-skeletal tissue—as well as its role in cancer. What the future holds for research on PTHrP also features largely in the conversation, an edited version of which appears below.

**BoneKEy:** What was your path to bone biology?

**Jack Martin:** After studying medicine at the University of Melbourne, I went for a post-doc at the Royal Postgraduate Medical School in London. Shortly after arriving there, I met up with Iain MacIntyre, who had just codiscovered calcitonin. It was a very exciting time! I managed to work out some *in vivo* experiments that enabled us to begin to study the mechanism of action of calcitonin—to show that it inhibited bone resorption. That was the start of my interest in calcium metabolism and its regulation. Following the calcitonin work, I came back to Melbourne and investigated parathyroid hormone (PTH), and then parathyroid hormone-related protein (PTHrP).

**BoneKEy:** What were the circumstances that led to your discovery of PTHrP?

**Jack Martin:** I had been interested for many years in the mechanisms of hypercalcemia in cancer, and worked on that subject throughout the 1970s, at first with my mentor in

Melbourne, Roger Melick. In the early 1970s, we had some evidence to suggest that PTH might be produced by certain hypercalcemic cancers, but the antibodies we used gave us conflicting data. Most of the antibodies didn't detect anything, and then other groups began to have very similar findings, suggesting that something other than PTH was being generated in this setting. I kept working in this area and developed an osteosarcoma cell line that generated, in rats, osteosarcomas that were remarkably PTH-responsive. The cells from those tumors provided the best bioassay for PTH activity that had ever been available.

By around 1980, the evidence had become very strong that whatever it was that caused the hypercalcemia of malignancy wasn't PTH, but rather something else that did very similar things—it increased cyclic AMP, it increased phosphate excretion, and it promoted bone resorption—but was chemically different from PTH. At that time, we found that a cell line I had been studying from other points of view, and that had been created from a hypercalcemic lung cancer patient, had in its medium an activity that stimulated cyclic AMP formation in the osteosarcoma cells. That activity was not influenced at all by a neutralizing antibody to PTH, but it was blocked by a receptor antagonist to PTH. That told us that it was worth purifying the activity in that medium, and that's what we did over the next several years. It was a major effort in protein purification to produce enough of the final activity in a pure form to obtain any sequence data; we had a number of people in the lab working on it, particularly Jane Moseley. In fact, it took us a bit over 4 years before we had enough material to sequence the protein.

**BoneKEy:** What was the attempt to sequence PTHrP like?

**Jack Martin:** The first time we obtained enough purified PTHrP to sequence in late 1985, it was something less than 5 picomoles. Today, if you have 5 picomoles of pure protein, it's a breeze to determine the sequence, but it wasn't so easy back then. My collaborator in Melbourne, Richard Wettenhall, was doing the sequencing, and with that very small amount of purified material he sequenced eight amino acid residues. About a year later we obtained a slightly larger amount of protein and sequenced the first 24 amino acids, revealing that 8 of the first 13 amino acids were identical to those of PTH.

By early 1987, we had also been trying for about 18 months to clone PTHrP—Larry Suva was my PhD student working on this—but we hadn't managed to do it. We then decided that we would do two things. First, we would publish the sequence that we had for a new protein that we called PTH-related protein. We submitted that work to *PNAS*<sup>1</sup> and presented it at the ASBMR meeting that year. In addition to the first 24 amino acids, the *PNAS* paper reported sequence similarities to PTH, the fact that antibodies could distinguish between PTH and PTHrP, and that the *PTHrP* gene was separate from the *PTH* gene.

The second thing we decided was that we had done all we could to clone PTHrP, and so I got together with a colleague at Genentech, to where I sent Larry Suva. With Bill Wood at Genentech, Larry made a new cDNA library, and the team there succeeded in cloning PTHrP, in the spring of 1987. Just a few days before the cDNA sequence was determined, we obtained another purified PTHrP preparation that moved us further along with the protein sequencing to residue 40 and some later tryptic peptides. All of that work was published in *Science* in 1987.<sup>2</sup> Soon after, we reported Bruce Kemp's synthesis of active PTHrP peptides in *Science*.<sup>3</sup>

**BoneKEy:** After the sequencing and cloning work, how did research evolve on PTHrP's function, to actually figure out what it did?

**Jack Martin:** Pretty quickly we had antibodies to PTHrP that could be used for immunolocalization, and one early finding was that PTHrP was present in the keratinocyte layer of the skin. It was also found to be present in blood vessels. For years it had been known that PTH promoted the dilatation of the arterial vasculature when it was injected into animals. Soon after PTHrP was discovered, it became obvious that PTHrP was produced around blood vessels, and that the effect of PTH that had been observed earlier was just a pharmacological reflection of the relaxing effect of locally produced PTHrP on the smooth muscle of blood vessels.

It had also been known, particularly through sheep experiments performed in England by my colleague, Tony Care, that in the mammalian fetus there was a PTH-like, but not PTH, biological activity in fetal plasma. We wondered whether that PTH-like activity could be due to PTHrP. That started us off on experiments with Tony where the parathyroid glands were surgically removed from fetal lambs, who were then allowed to go through to term. With the removal of the parathyroids, we saw greatly impaired transport of calcium across the placenta from mother to fetus, and we showed that it was PTHrP that promoted that transport.<sup>4</sup> That was another early finding on PTHrP function.

It was also apparent very early on that PTHrP's functions were mainly paracrine ones. There never really appeared to be any endocrine role for PTHrP, except in cancers in which PTHrP is produced in excess and subverts the normal calcium homeostatic mechanisms and causes hypercalcemia; in lactation, where we and others found PTHrP in the circulation of about 60% of lactating mothers, but not in nonlactating controls; and in fetal life, where, as I mentioned, PTHrP promotes calcium transport across the placenta. Except in those few cases, it is clear that either there is no circulating PTHrP, or that there are vanishingly low levels. In fact, PTHrP has never been convincingly measured in the circulation of normal postnatal human subjects. But there is a great deal of paracrine PTHrP activity in many tissues.

**BoneKEy:** Let's talk about one of those tissues of most interest to BoneKEy readers: What does PTHrP do in bone?

**Jack Martin:** There were two major sets of key studies that answered that question. Hank Kronenberg's group demonstrated the importance of PTHrP in bone and cartilage development through its effect on endochondral bone formation, and the group also showed that PTHrP production is regulated by Indian hedgehog. That work was a major contribution to our understanding of PTHrP in bone and cartilage.<sup>5</sup>

The second set of key studies was contributed by Andrew Karaplis, who made a PTHrP knockout mouse.<sup>6</sup> That knockout was neonatal lethal, because of a rib cartilage defect, and could be rescued by expressing PTHrP under the control of the collagen II promoter. Following that work, other abnormalities were found, including the failure of mammary gland development; that is an area that John Wysolmerski has done so much work on.<sup>7</sup>

What I find most interesting in the followup to Karaplis' work was that although the PTHrP homozygotes died neonatally, the heterozygotes lived, and they appeared to live normally, except that they lost bone. In 2005, the Karaplis lab reported results of an osteoblast-specific knockout of PTHrP, and found that the knockout mimicked the bone phenotype of the heterozygote; in other words, there was virtually an osteoporotic phenotype.<sup>8</sup> That really told us that PTHrP produced locally in bone is probably important in bone remodeling—that it can contribute to bone formation through its action on the osteoblast, and it can also stimulate resorption. It also told us that while PTH is used as an anabolic treatment, it's probably just reflecting the local role of PTHrP through the PTH receptor and its action in bone remodeling.

**BoneKEy:** With regard to PTHrP effects in bone, what are the things that we don't know yet, that you would really like to understand?

**Jack Martin:** I'd like to know whether what I just said a moment ago is actually true—that PTHrP is important as a local mediator in bone remodeling, because there are conflicting data from studies of PTHrP localization in bone. But if PTHrP is important locally in bone, I would also like to know how it is regulated. It's a molecule that's very susceptible to proteolytic breakdown. In fact, that was a terrible problem to deal with, in trying to express, purify and assay PTHrP.

**BoneKEy:** Looking beyond bone, PTHrP also plays a large role in cancer. How did research in that area progress?

**Jack Martin:** Originally, it was thought that humoral hypercalcemia of malignancy was confined to squamous cell cancers, neuroendocrine cancers, and renal cortical carcinoma. But in 1972, we published a paper in *BMJ*, where we described a case of hypercalcemic breast cancer.<sup>9</sup> We had a PTH antibody that seemed to be picking up something that was not parallel to standards, so that suggested maybe it was something other than PTH. In retrospect, it was probably PTHrP.

When we had our first PTHrP immunoassay, we found that hypercalcemic women with breast cancer and multiple metastases very commonly had quite high levels of PTHrP in the circulation,<sup>10</sup> and soon showed by immunostaining and *in situ* hybridization that breast cancers produced PTHrP.<sup>11</sup> We wondered whether that might be related to bone metastasis formation, so that prompted us to look at the localization of PTHrP in metastases. We found that, in unselected subjects, a high proportion of breast metastases to bone expressed PTHrP,

compared with metastases to the liver, lung and other soft tissues.<sup>12</sup> That led us to suggest that perhaps the production of PTHrP was a property of breast cancers in bone that allowed them to stimulate resorption and make the environment accommodating for continued tumor growth.

Greg Mundy's lab then took this up.<sup>13</sup> His group showed that you could enhance the establishment and growth in bone of a human breast cancer grown in a nude mouse by overexpressing PTHrP, and this could be blocked by using antibodies to PTHrP, or by inhibitors of bone resorption. That work really focused attention on how effective bone resorption inhibitors might be in bone metastasis.

**BoneKEy:** How strong is the evidence that PTHrP is a major player in bone metastasis formation?

**Jack Martin:** The evidence is pretty heavily dependent on mouse data, and on immunolocalization data showing the presence of PTHrP in advanced breast cancer with multiple lytic metastases. Blocking PTHrP is a treatment in experimental tumors, but in a clinical scenario, there has only been one attempt to develop a neutralizing monoclonal antibody against PTHrP that might be used in treatment, by a pharmaceutical company that didn't progress with the project; it's not clear exactly why. So this has never been comprehensively studied, and it remains an open question whether such an approach could be effective in humans.

We started a prospective clinical study in 1989 of more than 500 consecutive breast cancers that were surgically operated on.<sup>14,15</sup> Our prediction was that PTHrP in the primary tumor would be predictive of bone metastasis and poor outcome, but the reverse was true: expression of PTHrP in the primary tumor was an independent predictor of improved outcome—that is, of fewer metastases. That result is different from what we are finding about the effect of PTHrP in late cancer. In that setting, PTHrP made by tumor cells in the bone marrow stimulates bone resorption. But the clinical study suggests that perhaps there is a separate, earlier effect of PTHrP in breast cancer, by an unidentified mechanism, that might make the tumor less invasive and contribute to a less malignant phenotype.

There has been no other prospective study. There are two mouse studies,<sup>16,17</sup> but with conflicting data. The first, by David Thomas, was concordant with what we'd suggested from our clinical study. But more recently, Richard Kremer, Andrew Karaplis and their colleagues in Montreal used a different mouse breast cancer model in younger mice and found that knockout of PTHrP enhanced malignancy and increased the death rate. Then, last year, there was a GWAS study including a very large number of women, in various countries, looking for genes that could be linked to breast cancer.<sup>18</sup> Of the three genes identified by linkage, one was in a locus where the only known gene is *PTHrP*. This linkage work had no outcome data, so the picture is still unclear.

**BoneKEy:** Looking at the cancer and bone arena more generally, that is, beyond PTHrP *per se*, what does the research outlook look like—what needs to be done?

**Jack Martin:** The most important thing is to get better metastasis models. The one model that has been used the most in studying cancer metastasis to bone is the intracardiac injection model where cancer cells eventually get to bone and grow there. Though that model does allow one to study aspects of the mechanisms by which cancer cells invade and grow in bone, it's not really a metastasis model. What we need are good

models of actual metastasis that take us through the whole panoply of events that occur during that process. There is a mouse model of metastasis, called 4TI, but while there are useful findings coming from it, that model is in many respects far too malignant and invasive, and so it's not easy to manage.

In addition, PTHrP has been much of the focus in all of those experiments, but I think to the exclusion of other things that could be equally important, such as cytokines that are produced by tumors and are equally capable of promoting resorption, such as interleukin (IL)-11, and gp130 cytokines, including oncostatin M.

Also, the role of prostaglandins has largely been pushed aside over the years with all of these other things that offer themselves. So I think there is a lot more of very great interest that we can look forward to in the cancer and bone field.

**BoneKEy:** When you look at the path that PTHrP has taken, what lessons—for research or otherwise—do you take away from it?

**Jack Martin:** I wrote about this recently, with Laurie McCauley, in an article in *JBMR*, looking back at 25 years of PTHrP research.<sup>19</sup> There has been a lot of progress, but I think there could have been a lot more. PTHrP is an exceptionally interesting molecule, but we gave it a terrible name: parathyroid-related protein with the small 'r' in the middle of PTHrP. At the time we thought we could always change the name in due course, but you never change these things. The name may have deterred a lot of people who may have thought that they didn't want to work on PTH—but PTHrP is so different from PTH, and we have only scratched the surface. For instance, what about other biological activities of PTHrP? There is no doubt those other activities exist within the molecule, and they need to be sorted out. As another example, what does PTHrP do in the nucleus? Why does it have such a specific nuclear transport process? These are tremendously interesting questions for the future.

**BoneKEy:** Thank you so much for sharing the story of PTHrP with BoneKEy.

**Jack Martin:** It was my pleasure.

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