Abstract
Since its discovery 12 years ago, intensive research has been performed on ghrelin. The significance of ghrelin as a growth hormone-releasing hormone, appetite regulator, energy conservator and sympathetic nerve suppressor has now been well established. In this short essay, we summarize the history of the discovery of ghrelin.

Beginning
Everything has a beginning and an end. Sometimes a research ends with good results and sometimes it ends up as a failure. In the case of our ghrelin research, it ended with good results. If one of our team members, Kenji Kangawa, Hiroshi Hosoda, or I were not involved, ghrelin would not have been discovered. This short essay is a record of our research on ghrelin.

Our research style is ‘in the beginning there was a novel peptide’. We have been searching for novel unknown peptides for almost 30 years. We discovered the opioid peptides (α-neoendorphin, etc.), neuromedins and the natriuretic peptide family (ANP, BNP and CNP). It is very exciting to find a novel peptide and explore unknown physiological functions. It is just like a treasure hunt or climbing a virgin peak.

Kenji and I moved from Miyazaki Medical College to the National Cardiovascular Center Research Institute in Osaka in 1993 and began to search for novel peptide hormones. However, we could not find any novel peptides for the first 6 years, except for known peptides and fragments derived from nonpeptide hormone proteins. Hiroshi joined us as a graduate student to take a PhD degree just before we had tackled the search for the endogenous ligand to the GHS-R.

From the beginning of 1998, we had been searching for the endogenous ligands of several orphan GPCRs, such as GHS-R, BRS-3, GPR37, GPR39, mas, etc., although
none of the ligands except for ghrelin have yet been discovered. Among several of the orphan GPCRs that we searched, GHS-R (growth hormone secretagogue receptor) is somehow an exception [1]. Most of these orphan receptors did not have a specific activator; however, GHS-R had its specific activator GHSs, a group of synthetic compounds that stimulate GH release [2]. This means that the GHS-R assay system can be monitored.

**Stomach**

By January 1999, almost 1 year after we had started the ligand search for the GHS-R, we had found several peptides that activated the GHS-R; however, these peptides were always protein fragments, such as Purkinje cell protein 2 or myelin basic protein, and their activities were very low, indicating that these peptide fragments were not the real ligand for the GHS-R. We had undertaken many steps of chromatography and ran more than 500 assays and still had no hint of the ligand. We began to think that we should change the target from brain to other tissues.

At that time another orphan GPCR, GPR38, that shows high homology to GHS-R had already been known [3]. GPR38 was later identified as the motilin receptor [4]. In total deadlock, we began to think that because GHS-R and GPR38 have similar amino acid structures, their endogenous ligands should cross-react to the other receptor. Then, if we can find the GPR38 ligand, we can get some hint for the GHS-R ligand. Since GPR38 is highly expressed in the stomach and thyroid tissues, we began to assay GHS-R-expressing cells by using stomach extract. After the success of finding our ghrelin, we found that only our group had changed the target tissue and no other groups had tried stomach samples. This was very lucky for us, because content of ghrelin in the stomach was too high that every group should have succeeded to find the ligand, if only they tried to find it in the stomach.

**A Novel Peptide**

Unexpectedly, too high amounts of the endogenous ligand exist in the stomach. Several milligrams of the stomach extract is sufficient for detecting the activity. We only needed 10 days to complete the purification from 1 g of rat stomach tissue. However, amino acid sequence analysis of the purified ligand had given no signal at the third amino acid. From the cDNA analysis of the ligand, the unknown third amino acid was identified as a serine. We synthesized the ligand peptide and checked the activity, but there was none. We compared the purified and synthetic ligands and found that their elusion positions on HPLC are very different, indicating that they have a different structure. One possibility is that the serine residue at the third position is modified by an unknown molecule and this modification should be necessary for the activity.
What is the modification? From the data of the molecular weight of the purified peptide, we speculated that modification of structure of the third serine to be with n-octanoic acid. Mixture of the natural and synthetic n-octanoyl peptides gave a single peak on HPLC, which means that the two peptides had a perfectly matched elution time. Then, we checked the GHS receptor-expressing cells to see whether the synthetic peptide had activated the cells. The activity of the synthetic peptide on the GHS receptor matched that of the natural peptide. Moreover, the synthetic and natural peptides showed the same profiles by their physical and chemical characters. Finally, we identified the structure as an acyl-modified peptide from the stomach [5]. We named this peptide 'ghrelin' derived from 'ghre', which means 'grow' in Indo-European roots. To our joy, the name is accepted by the research world.

After the Discovery of Ghrelin

After the discovery of ghrelin, we and other groups examined the physiological functions of ghrelin and found that ghrelin is a potent orexigenic hormone [6–8]. This was very exciting since ghrelin is a circulating hormone and these results indicated a future application of ghrelin for the treatment of eating disorders.

Another interesting point on ghrelin was the identification of the enzyme, ghrelin O-acyltransferase (GOAT), which modified and activated ghrelin. We had searched for the enzyme since the discovery of ghrelin. However, in 2008 two other groups reported the enzyme and we lost [9, 10]. The results were very surprising and exciting, because the enzyme was exclusively specific for the acyl modification of ghrelin. Thus, several inhibitors for GOAT may be useful for the treatment of metabolic disorders [11].

Moreover, it remained to be answered whether ghrelin is the only acyl-modified peptide hormone or not, because several orphan acyltransferases whose substrates have not yet been identified are registered in the genome database [12].

Recent developments in research technology enable us to challenge difficult questions in ghrelin research. For example, the crystal structure of the ghrelin receptor and 3-D analysis of the receptor activation will be characterized in the near future. How does the n-octanoyl moiety of ghrelin bind to the ghrelin receptor and why is the acyl modification necessary for receptor activation? These questions will be answered by the latest methods for determining the crystal structures of membrane proteins.

Epilogue

The discovery of ghrelin changed our (Kojima, Hiroshi and Kenji) lives, of course to a better one. However, even in these days I often imagine what would have happened in my research life if I had not changed the target tissue from brain to stomach or if I
could not have solved the modified structure of ghrelin. We need some luck to get through to a good research. Although it may be a hackneyed phrase that whether you can get lucky or not depends on your passion for your research, I believe it.

Lastly, please let me express my personal pleasure. I am very very happy and honored to find that ‘ghrelin’ is described in Lehninger’s Biochemistry and Stryer’s Biochemistry, textbooks that I used for study in biochemical courses in my college student days. I could not have imagined when I was a young student that a discovery of mine would be in these textbooks 30 years later.

References