Genomics and proteomics of the chromaffin cell: characterization of cell differentiation and chromogranin peptide formation.

Youssef Anouar[a], Luca Grumolato[a], Djida Ait-Ali[a], Maité Montero-Hadjadje[a], Laurent Yon[a], Johann Guillemot[a], Valérie Turquier[a], Abdel G. Elkahloun[b], Lee E. Eiden[c] and Hubert Vaudry[a]

a. European Institute for Peptide Research, Laboratory of Cellular and Molecular Neuroendocrinology, INSERM, UA CNRS, University of Rouen, France.
b. Cancer Genetics Branch, NHGRI, NIH, Bethesda.
c. Section on Molecular Neuroscience, Laboratory of Cellular and Molecular Regulation, NIMH, NIH, Bethesda, USA.

Correspondence: Dr. Youssef Anouar, European Institute for Peptide Research (IFRMP 23), Laboratory of Cellular and Molecular Neuroendocrinology, INSERM U413, UA CNRS, University of Rouen, 76821 Mont-Saint-Aignan, France.
Phone: 33-235-14-6661; **Fax:** 33-235-14-6946; **Email:** youssef.anouar@univ-rouen.fr

Cell Biology of the Chromaffin Cell
R. Borges & L. Gandía Eds.
Instituto Teófilo Hernando, Spain, 2004

Chromaffin cells of the adrenal medulla have been widely used to decipher the mechanisms of neurotransmitter action as well as hormone biosynthesis and exocytosis. Moreover, the chromaffin cell-derived pheochromocytoma PC12 cells represent a useful model for studying neuronal and neuroendocrine cell regulation and differentiation in the sympathoadrenal lineage. We took advantage of the technological advances in the fields of genomics and proteomics, which allow the integration of a large amount of gene expression information in a unique and global view, to gain insight into the genetic program that governs sympathoadrenal cell differentiation. For this purpose, we compared the transcriptomes of undifferentiated rat pheochromocytoma PC12 cells and terminally differentiated rat adrenomedullary cells [1]. This analysis allowed the identification of more than one thousand differentially expressed genes (Fig. 1, A and B), including a large number of factors involved in cell proliferation that are mainly overexpressed in PC12 cells, *i.e.* components of mini-chromosome maintenance deficient complex or pituitary tumor transforming 1. On the contrary, the majority of the genes involved in cell adhesion and ion transport were more highly expressed in chromaffin cells, *i.e.* biglycan or ferredoxin reductase. It is worth noting that an important group of the identified genes that are differentially expressed between chromaffin cells and PC12 cells is associated with apoptosis and protein processing, *i.e.* proteasome subunits or glutathione peroxidases. In order to better understand the transcriptional events that accompany the differentiation of sympathoadrenal cells, we studied the modifications of the gene expression profile of PC12 cells following treatment with the neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP), a major regulator of adrenal activity [2] which is able to functionally differentiate these cells [3,4]. Microarray and subtractive hybridization analyses identified 145 genes regulated by PACAP in PC12 cells (Fig. 1, C and D), a large proportion of which are involved in cell proliferation and signaling, *i.e.* B-cell translocation gene 2 or bone morphogenetic protein 6. PACAP also regulated the expression of several genes associated with cell survival, *i.e.* Bcl2-associated athanogene 3, and motility/adhesion, *i.e.* kinesin-like 5 and embigin. Comparison of the transcriptome alterations in PC12 cells versus

adrenomedullary cells and undifferentiated PC12 cells versus PACAP-treated PC12 cells revealed that the majority of the common genes identified in both analyses are in fact more actively expressed in tumoral chromaffin cells and down-regulated by PACAP. These are mainly factors involved in cell proliferation, which are likely to be involved in the differentiation of sympathoadrenal cells during development.

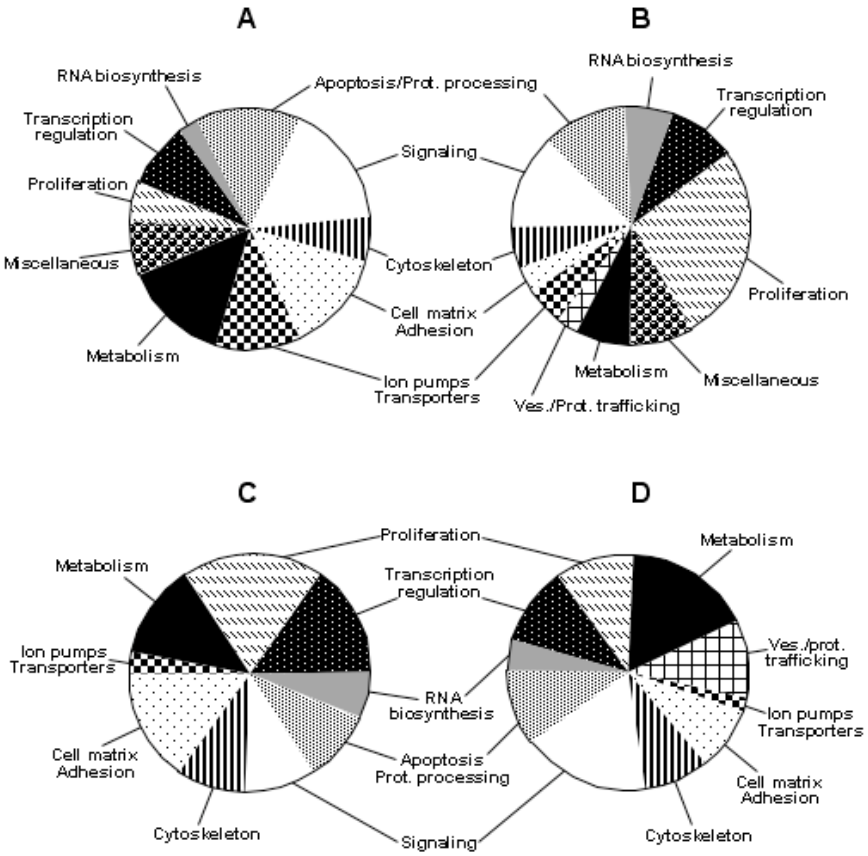

Figure 1. Functional clustering of the genes differentially expressed between rat adrenomedullary cells, PC12 cells and PACAP-treated (100 nM, 48 hrs) PC12 cells. A) genes over-expressed in adrenomedullary cells compared to PC12 cells. **B)** genes over-expressed in PC12 cells compared to adrenomedullary cells. **C)** genes down-regulated by PACAP in PC12 cells. **D)** genes up-regulated by PACAP in PC12 cells.

Among the genes differentially expressed in the three cell models studied, we identified various members of the chromogranin family of proteins which play a role in the acquisition and the function of the secretory phenotype [5].

The high homology of discrete sequences of chromogranins, that are delimited by basic residues whereas the rest of the proteins is poorly conserved in phylogenetically distant species, strongly suggests that these proteins are precursors of potentially active peptides [6,7]. In order to investigate the biological relevance of these conserved regions of chromogranins in sympathoadrenal cells, we first demonstrated by immunohistochemistry and HPLC analysis the occurrence of the peptides EL35 and WE14, which derive from chromogranin A, and EM66, which originates from secretogranin II processing, in both fetal and adult human adrenal gland. The possible implication of these peptides in pathological processes is supported by the presence of EM66 in various pheochromocytoma samples. Interestingly, a significant difference in EM66 concentration was observed between benign and malignant tumors, suggesting that measurement of EM66 levels may be of clinical value for the prognostic of pheochromocytoma progression [8-10].

In conclusion, genomics and proteomics approaches have been applied to identify genes and peptides associated with normal and tumoral chromaffin cells, in order to provide new insight in the molecular mechanisms involved in sympathoadrenal cell differentiation in normal and pathological condition. We are currently focusing on the functional characterization of the identified genes and peptides in this cell lineage.

REFERENCES

1. Grumolato, L., et al., *Microarray and suppression subtractive hybridization analyses of gene expression in pheochromocytoma cells reveal pleiotropic effects of pituitary adenylate cyclase-activating polypeptide on cell proliferation, survival, and adhesion*. Endocrinology, 2003. **144:**2368-2379.
2. Hamelink C, Weihe E. and L.E. Eiden. *PACAP: an "emergency response" co-transmitter in the adrenal medulla.* In *Pituitary Adenylate Cyclase-Activating Polypeptide*. H Vaudry & A Arimura Eds: 227-249. Kluwer Academic Publishers. Norwell, MA. 2003.

3. Deutsch, P.J. and Y. Sun. *The 38-amino acid form of pituitary adenylate cyclase-activating polypeptide stimulates dual signaling cascades in PC12 cells and promotes neurite outgrowth*. J Biol Chem, 1992. **267:**5108-5113**.**
4. Grumolato, L., et al., *PACAP and NGF regulate common and distinct traits of the sympathoadrenal lineage: effects on electrical properties, gene markers and transcription factors in differentiating PC12 cells*. Eur J Neurosci, 2003. **17:**71-82.
5. Kim, T., et al., *Chromogranin A, an "on/off" switch controlling dense-core secretory granule biogenesis*, Cell, 2001. **106:**499-509.
6. Turquier, V., et al., *Characterization of chromogranins in the frog Rana ridibunda. Structure, expression, and functional implications*. Adv Exp Med Biol, 2000. **482:**125-136.
7. Ait-Ali, D., et al., *Molecular characterization of frog chromogranin B reveals conservation of selective sequences encoding potential novel regulatory peptides*. FEBS Lett, 2002. **511:**127-132.
8. Montero-Hadjadje, M., et al., *Localization and characterization of evolutionarily conserved chromogranin A-derived peptides in the rat and human pituitary and adrenal glands*. Cell Tissue Res, 2002. **310:**223-236.
9. Montero-Hadjadje, M., et al., *Biochemical characterization and immunocytochemical localization of EM66, a novel peptide derived from secretogranin II, in the rat pituitary and adrenal glands.* J Histochem Cytochem, 2003. **51:**1083-1095.
10. Yon, L., et al., *Identification of the secretogranin II-derived peptide EM66 in pheochromocytomas as a potential marker for discriminating benign versus malignant tumors.* J Clin Endocrinol Metab, 2003. **88:**2579-2585.