Do Transcription Factors Hold the Key to Understanding the Development of Barrett's Esophagus?

Rebecca C. Fitzgerald

Received: 17 January 2012 / Accepted: 18 January 2012 / Published online: 25 February 2012
© Springer Science+Business Media, LLC 2012

Barrett's esophagus is a complex metaplasia which can occur as a pure gastric or intestinal histopathological phenotype but more commonly occurs as a mosaic comprising gastric and intestinal forms. Intestinal metaplasia is typified by the presence of goblet cells seen on light microscopy. Some studies indicate that the chances of identifying goblet cells are related to the length of Barrett's, location of the biopsies (more goblet cells in proximal esophagus), number of biopsies, patient age and gender, being more common in men [1]. Most interest has focussed on those patients with an endoscopically visible columnar lined epithelium containing intestinal metaplasia since this is the form most clearly associated with malignancy with an annual progression rate estimated to be in the region of 0.3–0.4% per annum [2, 3]. However, recent studies comparing goblet and non-goblet containing metaplastic epithelium have shown that both types demonstrate the same frequency of DNA content abnormalities [4, 5], suggesting that the gastric form is not entirely benign.

The molecular and cell biological process by which Barrett's esophagus arises is poorly understood. The emerging theories include trans-differentiation from a mature esophageal keratinocyte or altered differentiation of an esophageal or submucosal stem cell [6]. More recently it has been suggested that Barrett's may occur from expansion of an embryological remnant [7]. Whichever theory is correct, all appear to depend on the activation of transcription factors to alter the cell fate. To date most effort has focussed on the homeobox genes *Cdx1* and *2* which, in contrast to the dramatic effect observed when ectopically

expressed in the murine stomach [8], fail to induce intestinalisation when over-expressed in the esophagus. Other transcription factors examined have included Hedgehog [9] and another critical signalling molecule in embryonic development called BMP4 [10], which have generally failed to induce phenotypic intestinalisation and in particular goblet cells.

The aim of this paper was therefore to explore whether the helix-loop-helix transcription factor *Math1/Atoh1*, which is required for the differentiation of the three secretory cell lineages in the intestine (enteroendocrine, Paneth and goblet cells), could induce intestinalisation of an esophageal cell line. In order to explore this Kong et al. [11] first confirmed earlier reports that this gene is over-expressed in Barrett's esophageal tissues [12]. RNA expression levels in endoscopic biopsies were found to be at a highly variable level (from 10- to 1,000-fold), but the levels were always in excess of that found in paired normal esophageal biopsies. It is unfortunate that no matching histology was available for these Barrett's samples, since as noted in the discussion, it is likely that this variation in RNA expression levels correlated with the goblet cell numbers which are known to be heterogeneous in this epithelium. This is a methodological point worth noting since many studies in the Barrett's field are hampered by lack of good pathological correlations relying instead on paired biopsies or information from H&E sections taken elsewhere in the Barrett's segment. Frozen sections from the same sample that is being used for molecular analyses can provide invaluable information, given that intestinalisation and dysplasia can be highly focal, and with advancing technology this does not preclude sufficient material for most assays.

The bulk of the paper by Kong et al. [11] utilised a telomerase immortalised human esophageal keratinocyte line (EPC-hTERT) grown in a 3D organotypic culture

R. C. Fitzgerald (✉)
MRC Cancer Cell Unit, Hutchison-MRC Research Centre,
Cambridge, UK
e-mail: rcf@hutchison-mrc.cam.ac.uk

model. By utilising a 3D culture system it is easier to explore the functional significance and morphology of the experimental conditions. The authors used retroviral infection to create two distinct lines overexpressing *Math1/Atoh1*. This led to a reduction in cell proliferation, and 20% of transfected cells had a more elongated and branching morphology compared with un-transfected control cells. Most interestingly, two genes Mucin-2 and Keratin-20 associated with an intestinal phenotype were up-regulated at the RNA and to some extent at the protein level. However, these alterations in gene expression were insufficient to induce goblet cell differentiation or to lead to the production of intestinal mucins.

The focus on Muc-2 here is interesting since the non-goblet containing epithelium has also been shown to have expression of this protein as well as other intestinal immunohistochemical markers such as CDX-2, Hep Par 1, DAS-1 and villin [7, 13].

It is increasingly recognised that multiple transcription factors are required to alter cell fate. Prior to this paper, the research group led by John Lynch had been working on combinations of transcription factors to induce intestinalisation. For example, Cox2 activity and Cdx1/c-Myc co-expression studies have led to mucin production and alcian-blue staining without Muc-2 expression. Hence, as proposed by the authors, combining *Math1/Atoh1* together with Cox2 and so on may lead to a more complete intestinalisation. Furthermore, it may be necessary to limit the squamous cell self-renewal as seen, for example, by development of a Barrett's-like state in mice null for the p63 gene, which is the master transcriptional regulator for squamous epithelia [7]. A further factor which may be contributing to the difficulty in obtaining an overt intestinal phenotype is the derivation of the "originator" cell used for these experiments. For example, use of telomerase immortalised esophageal keratinocytes may be a stumbling block to achieving a more complete alteration in cell fate. In vivo models suggest that established columnar cells may be more easily differentiated into post-mitotic goblet cells [14] and hence a multi-step approach may be required.

The origin of Barrett's esophagus continues to intrigue and perplex scientists but clues are emerging from work on a growing number of transcription factors which, combined with our growing ability to isolate the relevant possible cells of origin and to work with more physiologically relevant model systems, will hopefully bring us nearer to the truth.

Key Points

- Transcriptional regulators such as *Math1/Atoh1* contribute to determination of cell fate relevant to Barrett's metaplasia.

- Multiple transcription factors are likely to be required to result in overt intestinalisation.
- The cell or origin and hence the model system used may contribute to the disappointing results seen in attempts to alter cell fate in vitro.
- Barrett's researchers should aim wherever possible to obtain histopathological correlates for molecular analyses through the use of frozen sections or in situ techniques for measuring gene expression to aid accurate interpretation of data.

Conflict of interest None.

References

1. Harrison R, Perry I, Haddadin W, et al. Detection of intestinal metaplasia in Barrett's esophagus: an observational comparator study suggests the need for a minimum of eight biopsies. *Am J Gastroenterol*. 2007;102:1154–1161.
2. Desai TK, Krishnan K, Samala N, et al. The incidence of oesophageal adenocarcinoma in non-dysplastic Barrett's oesophagus: a meta-analysis. *Gut*. 2011. doi:10.1136/gutjnl-2011-300730.
3. Bhat S, Coleman HG, Yousef F, et al. Risk of malignant progression in Barrett's esophagus patients: results from a large population-based study. *J Natl Cancer Inst*. 2011;103:1049–1057.
4. Odze RD. Barrett esophagus: histology and pathology for the clinician. *Nat Rev Gastroenterol Hepatol*. 2009;6:478–490.
5. Liu W, Hahn H, Odze RD, Goyal RK. Metaplastic esophageal columnar epithelium without goblet cells shows DNA content abnormalities similar to goblet cell-containing epithelium. *Am J Gastroenterol*. 2009;104:816–824.
6. Barbera M, Fitzgerald RC. Cellular origin of Barrett's metaplasia and oesophageal stem cells. *Biochem Soc Trans*. 2010;38:370–373.
7. Wang X, Ouyang H, Yamamoto Y, et al. Residual embryonic cells as precursors of a Barrett's-like metaplasia. *Cell*. 2011;145:1023–1035.
8. Mutoh H, Hakamata Y, Sato K, et al. Conversion of gastric mucosa to intestinal metaplasia in Cdx2-expressing transgenic mice. *Biochem Biophys Res Commun*. 2002;294:470–479.
9. Wang DH, Clemons NJ, Miyashita T, et al. Aberrant epithelial-mesenchymal Hedgehog signaling characterizes Barrett's metaplasia. *Gastroenterology*. 2010;138:1810–1822.
10. Milano F, van Baal JW, Buttar NS, et al. Bone morphogenetic protein 4 expressed in esophagitis induces a columnar phenotype in esophageal squamous cells. *Gastroenterology*. 2007;132:2412–2421.
11. Kong J, Crissey MAS, Sepulveda AR, Lynch JP. *Math1/Atoh1* contributes to intestinalisation of esophageal keratinocytes by inducing the expression of *Muc2* and *Keratin-20*. *Dig Dis Sci*. 2011. doi:10.1007/s10620-011-1998-y.
12. Chen X, Qin R, Liu B, et al. Multilayered epithelium in a rat model and human Barrett's esophagus: similar expression patterns of transcription factors and differentiation markers. *BMC Gastroenterol*. 2008;8:1.
13. Hahn HP, Blount PL, Ayub K, et al. Intestinal differentiation in metaplastic, nongoblet columnar epithelium in the esophagus. *Am J Surg Pathol*. 2009;33:1006–1015.
14. Menke V, van Es JH, de Lau W, et al. Conversion of metaplastic Barrett's epithelium into post-mitotic goblet cells by gamma-secretase inhibition. *Dis Model Mech*. 2010;3:104–110.