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Mini-review

Anterior gradient 2: A novel player in tumor cell biology

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ABSTRACT

AGR2 has evolutionarily conserved roles in development and tissue regeneration and is linked with several human cancers. The exact functions and regulation of AGR2 are poorly understood, but current data identify AGR2 as a clinically relevant factor that modulates the behavior and response of hormone-dependent cancers (breast, prostate) and hormone-independent cancers (colorectal, pancreatic, esophageal and other common cancers). AGR2 protein expression induces metastasis, acts as a p53 tumor suppressor inhibitor and survival factor, participates directly in neoplastic transformation and is involved in drug resistance. Thus, AGR2 is an important tumor biomarker and negative prognostic factor potentially exploitable in clinical practice.

1. Introduction

Anterior gradient (AG) genes were first identified in *Xenopus laevis* and are named according to their specific expression patterns during early development [1]. Three AG transcripts were discovered by dissection of variously aged embryos of *X. laevis*. XAG-1, XAG-2 and XAG-3 are all approximately 2 kb in length and are first expressed in the anterior region of the dorsal ectoderm in late gastrula embryos [1,2]. The temporal and spatial pattern of XAG-2 expression during amphibian embryogenesis corresponds to the anterior region of the dorsal ectoderm at late gastrula and later the expression gradually becomes restricted to the anterior-most part of the dorsal ectoderm. At the onset of neurulation XAG-2 mRNA is located at the anterior border between the dorsal and ventral ectoderm. This region corresponds to the cement gland anlage, where XAG-2 transcripts are exclusively expressed in successive stages of the frog embryo [3]. The activation of XAG-2 expression is under the control of neuralizing signals including the noggin, chordin, follistatin and cerberus molecules emanating from the organizer region [3]. XAG-2 has been reported to be a secretory protein and a signal peptide was identified in the first 18 amino acid residues [3,4].

Strikingly, the newt AG homolog (nAG) was the first protein ever discovered with the ability to promote limb regeneration [5]. Newt limbs regenerate from de-differentiated fibroblast, muscle, skeletal and Schwann cells into their earlier stem cell forms. These stem cells then proliferate to produce a mass of undifferentiated cells in a process that requires factors for proliferation and overall regeneration. nAG protein is a mitogen for newt blastema cells and also a secreted ligand for Prod-1 protein. During early stages of de-differentiation, nAG was detected in Schwann cells of the distal nerve sheath, later on it was found to be expressed in gland cells of the wound epidermis [5].

Recently, another homologous protein to the anterior gradient secretory protein family was discovered in Xenopus embryo. This protein, named XAgr2, is strongly expressed in the cement gland [6] and other ectoderm-derived organs such as otic vesicles and notochord during neurula and tailbud stages of frog embryogenesis [7]. Interestingly, XAgr2 has greater sequence homology to the human AGR2 or mouse Agr2 (MAgr2) genes than to XAG-1 and XAG-2 genes of *X. laevis*. Anterior gradient proteins have been identified in zebrafish *Danio rerio*

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Table 1
AGR2 expression in normal tissues (derived from Human Protein Atlas, http://www.proteinatlas.org).

Normal tissues IHC[a]	Cell type	Protein expression
Appendix	Glandular cells	Strong
Breast	Glandular cells	Weak
Bronchus	Respiratory epithelial cells	Strong
Cerebral cortex	Neuronal cells	Moderate
Cervix, uterine	Glandular cells	Strong
Colon	Glandular cells	Strong
Corpus, uterine	Glandular cells	Strong
Duodenum	Glandular cells	Strong
Epididymis	Glandular cells	Strong
Fallopian tube	Glandular cells	Strong
Gall bladder	Glandular cells	Strong
Kidney	Cells in tubules	Moderate
Lung	Alveolar cells	Moderate
Nasopharynx	Respiratory epithelial cells	Strong
Pancreas	Exocrine glandular cells	Moderate
Placenta	Trophoblastic cells	Moderate
Prostate	Glandular cells	Strong
Rectum	Glandular cells	Strong
Seminal vesicle	Glandular cells	Strong
Small intestine	Glandular cells	Strong
Stomach	Glandular cells	Strong
Tonsil	Squamous epithelial cells	Moderate
Urinary bladder	Urothelial cells	Strong

[a] Immunohistochemical staining.

(Zagr2), pufferfish *Tetraodon nigroviridis* (Tagr2), Atlantic salmon Salmo salar (Sagr2) and in mammals including human AGR2 (AG2, GOB_4, HAG-2, PDIA17, XAG-2) and AGR3 or MAgr2 and mouse Agr3 (MAgr3) [6]. In contrast to *X. laevis*, where anterior gradient proteins are mainly expressed in ectoderm-derived organs, human and mouse anterior gradient proteins are mainly distributed in endoderm-derived organs [8].

Interestingly, phylogenetic studies have revealed several branches in the AG protein family tree. These studies suggest closer relationships between human AGR3, MAgr3 and Xenopus XAG/1/2 genes, and the similarity within the fish branch with human AGR2 and MAgr2 genes [6].

2. Human AGR2

AGR2 and AGR3 are the two human homologs of the originally discovered *X. laevis* secreted protein XAG-2. Human AGR2 is strongly expressed in the lung, stomach, colon, prostate and small intestine – tissues that contain mucus secreting cells and/or function as endocrine organs [6,9] (Table 1). From an evolutionary perspective, AGR2 may be involved in the epithelial barrier function, because AGR2 promoter was found to be regulated by transcriptional factors typical for epithelial goblet cells [10].

The gene for human AGR2 lies at chromosomal position 7p21.3 and shares 71% identity with its homolog AGR3 [11]. AGR2 gene consists of eight exons and nine transcripts in total have been described for this gene. However only six of them, AGR2 001, AGR2 005, AGR2 006, AGR2 007, AGR2 201 and AGR2 202 are protein coding. These splice variants of the AGR2 protein are within the range of 119 and 188 amino acids length (Fig. 1). The most abundant variant, AGR2 001, consists of 175 amino acids with a predicted molecular weight of 19,979.2 Da and a predicted pI value of 9.03. The alignment of the protein sequence of AGR2 revealed 54% identity and 71% similarity with XAG-2, and 91% identity and 96% similarity with MAgr2 [4].

The AGR2 protein contains a hydrophobic endoplasmic reticulum leader sequence with a signal peptidase cleavage site at Ala20/Lys21 and also has a retention sequence in the C-terminus that is likely to effect intracellular trafficking [12].

With regard to taxonomy, AGR2 is a member of the protein disulfide isomerase (PDI) family of endoplasmic reticulum-resident proteins [13]. These proteins contain one to four CXXC active domain motifs facilitating oxidative/reductive reactions, which are also found in thioredoxins (Tx) and thus referred as Tx-domain motifs. Several PDIs including AGR2 contain thioredoxin-like domain CXXS, which exhibits lower activity associated with disulfide bond reorganization compared to the CXXC domain, nevertheless they may contribute to isomerization of disulfide bridges and possibly perform other specialized functions in the endoplasmic reticulum [13].

The fundamental role of these motifs with active cysteine residues is to form mixed disulfide bonds with substrates. AGR2 itself has recently been shown to form mixed disulfides with intestinal mucin. AGR2 is present within the endoplasmic reticulum of intestinal secretory epithelial cells and interacts with Mucin 2 (Muc2), a major component of intestinal mucus, enabling the large number of Muc2 Cys residues to pair correctly as it is processed by mucus-producing cells. A structural relationship involving putative functional domains between AGR2 and proteins of the disulfide isomerase family of molecular chaperones suggests a role in protein folding [14].

In our recent work we identified an ATP binding protein, Reptin, as a protein that interacts with AGR2 in a yeast two-hybrid screen and validated as an AGR2 binding protein in human cells. We found that the PDI thioredoxin domain of AGR2 is not directly involved in binding to Reptin, but we detected a specific domain that can bind stably to Reptin, and this was localized by mutagenesis within amino acids 104–111 on the AGR2 primary sequence [15]. This region represents a divergent loop, which apart from AGR2 and AGR3 was also found in Erp18 (the smallest member of PDI family) and has been proposed to represent substrate-binding sites for the molecular chaperone function of the protein [16,17]. Accordingly, this loop insertion forms the minimal docking site for Reptin within AGR2 and in turn probably regulates Reptin's many functions such as ATPase activity, ATP binding, helicase functions, telomerase/Pontin binding, APPL1/2 binding, TIP60 interactions, and other related protein signaling functions.

3. AGR2 biological function

Although the role of AGR2 at the level of cells or organisms has not been fully described in mammals, some speculations about their possible functions may be derived from the literature referring to the Xenopus homologs XAG-1 and XAG-2 [9].