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Review Article

From development to cancer - an ever-increasing role of AGR2

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Abstract: Anterior gradient 2, AGR2, is a small, 20 kDa protein that plays a vital role in oxidative protein folding in the endoplasmic reticulum. AGR2 is involved in several signal transduction pathways that are essential for cell survival. It was initially discovered in the African clawed frog, *Xenopus laevis*, where it plays an important function in embryonic development. Akin to several other developmental genes, it is also frequently deregulated in cancer, where it plays a decisive role in tumor initiation, progression and metastasis. In this review, we have summarized currently known AGR2 functions, its expression and function in embryonic and cancer development, as well as its potential as a candidate tumor biomarker and promising new target for cancer immunotherapy.

Keywords: AGR2, anterior gradient 2, embryonic development, cancer, tumor biomarker, therapeutic target

Introduction

Anterior gradient 2 (AGR2) is a 154 amino acid protein disulfide isomerase (PDI) with a single central cysteine residue [1]. It is a resident endoplasmic reticulum (ER) protein highly expressed in mucus-secreting cells and human endocrine tissues [1, 2]. The *AGR2* gene belongs to a small family of *AGR* genes (*AGR1, 2* and *3*), and is localized on chromosome 7p21.3 in the human genome [3]. It plays an important role in embryonic development [4, 5] and tissue regeneration [6] and has been linked with the initiation and progression of several cancer types [7-12].

In this review, we illustrate the expression of AGR2 in the embryonic development of several species, including human, summarize its role across normal and diseased states, and describe its promising function in the detection and treatment of cancer.

AGR2 as a developmental gene

AGR2 was initially identified in the African clawed frog, *Xenopus laevis*, where it is expressed in the anterior region of the ectoderm and plays an important role in the ectodermal patterning and formation of anterior ectodermal structures such as cement gland and early forebrain [4, 5]. The cement gland is the mucus-secreting organ required for embryo attachment to a solid support before the development of swimming and feeding abilities [13]. AGR2 has also been shown to be involved in the regeneration of the limbs and the tail in frog tadpoles [6].

AGR2 was detected in mucus-secreting organs of other species as well. In embryos of zebrafish Danio rerio, it is required for the terminal differentiation of mucin-producing goblet cells [14, 15]. In the adult fish, it is expressed in organs containing mucus-secreting cells including gill, esophagus, swim bladder, and intestine [15].

In mice, *Agr2* gene plays an important function in intestinal mucus production and gut protection, as mice lacking AGR2 have an increased risk of the development of colitis [2]. Moreover, the loss of AGR2 expression causes hyperplasia and defective lineage maturation in AGR2

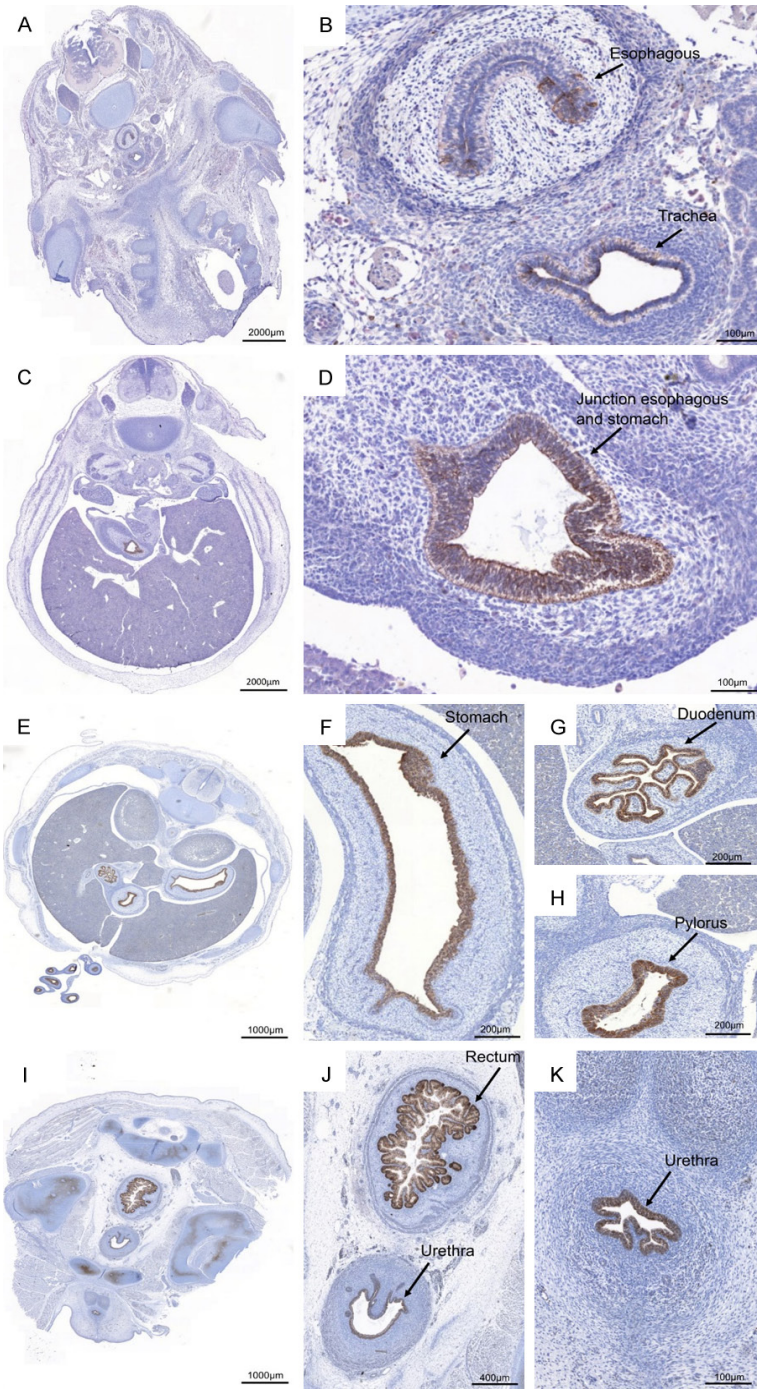

Figure 1. AGR2 expression in the gastrointestinal tract of the human embryo. The human embryonic and fetal material from 3 to 20 weeks of development was obtained from the MRC-Welcome Trust Development Human Developmental Biology Resource (HDBR) tissue bank. Two sections of the human embryo and fetal tissues were used for each stage from Carnegie Stage 19 (CS) of embryonic development up to post-conception week 14 (PCW14). The AGR2 antibody was optimized for Formalin-Fixed Paraffin-Embedded (FFPE) tissues. The immunostaining was performed, and the pictures were scanned using Panoramic 250 3D Histech. AGR2 was found to be expressed across the gastrointestinal tract in several stages of embryonic development: in esophagus and trachea in CS19 (A, B); in the junction of esophagus and stomach; in CS20 (C, D), herniated intestines, stomach, duodenum, and pylorus in CS23 (E-H); and rectum and urethra in PCW10 (I-K).

null mouse model, indicating that AGR2 plays a role in maintaining the proliferation of glandular stomach cells [16]. Similarly, the loss of AGR2 expression leads to loss of tissue regeneration ability in the mouse pancreatitis model [17]. The AGR2 has been shown to regulate cellular proliferation and lobuloalveolar development in the mammary gland in mice, with the highest AGR2 expression observed during late pregnancy and lactation [18].

In this study, we demonstrate for the first time that AGR2 is also expressed during human embryonic development (**Figure 1**). The human embryonic period is divided into 23 Carnegie stages, based on the external and internal morphological development of the embryo, covering the first 8 weeks post-ovulation. AGR2 expression was first observed in embryos in Carnegie stage 19 (CS19), week 7 of embryo development onwards, initially in the esophagus, trachea, liver, and stomach (**Figure 1A**, **1B**). Its expression persisted in later stages in the gastrointestinal tract, where it was observed in the esophagus and stomach in CS20 (**Figure 1C**, **1D**) and duodenum, stomach, pylorus and herniated intestines in CS23 (**Figure 1E-H**). In the post-conception stage, AGR2 expression was also observed in the rectum in post-conception weeks (PCW) 9, 10, 12 and 14, and anus in PCW10 (**Figure 1I-K**).

In addition to the gastrointestinal tract, AGR2 expression was detected in the pigmented layer of the retina in CS22 (Supplementary Figure 1A, 1B) and a skeletal system in ilium (PCW9 and PCW12), ischium

(PCW12), pubis (CS23 and PCW10), head of the femur (CS23 and PCW10) and vertebral body (PCW10) (Supplementary Figure 1C-H) as well as in the urogenital system, including ureter (PCW9 and PCW14), urethra (PCW10) and bladder (PCW9 and PCW14), (Supplementary Figure 1I-K).

In adult human tissues, the highest level of AGR2 expression was observed in the gastrointestinal tract (from the stomach to rectum) and genitourinary tract (urinary bladder and female and male genitalia), as well as in respiratory epithelia of nasopharynx and bronchus [19].

Since Virchow's notion in 1840 that new formations (neoplasms) arise "in accordance with the same law, which regulates embryonic development" [20], abundant evidence was amassed that a number of pathways involved in embryonic and fetal development are later on re-utilized in both the control of postnatal cell growth and differentiation as well as in oncogenesis [21-23]. AGR2 is one of the genes that corroborate this association between embryonic development and cancer.

AGR2 in health and disease

AGR2 is a member of the protein disulfide isomerase (PDI) superfamily [24], which is an expanding family of enzymes that are predominantly expressed in the ER. Structural characteristics of PDI proteins include a carboxyl-terminal KTEL motif as well as at least one thioredoxin-like structural domain with a CXXS motif [11, 24-28]. The main function of the PDI family, including AGR2, is the regulation of protein folding. Under stress conditions, AGR2 activates the unfolded protein response (UPR) pathway in the ER, which is one of the key cellular defense mechanisms. AGR2 expression is commonly elevated during ER stress and directly controlled by ER signaling [29]. Accordingly, the siRNA-mediated knockdown of AGR2 can inhibit the ER-associated protein degradation process and disable cellular response to the ER stress [30].

The unstructured N-terminal region of AGR2 is responsible for the cellular adhesion, while the folded domain is essential for dimerization via intermolecular salt bridges [31]. The AGR2 dimerization process in ER can be inhibited by the ER stress, resulting in secretion of AGR2 monomers and activation of pro-inflammatory responses. This can lead to the development of Crohn's and inflammatory bowel disease (IBD). Interestingly, the level of dysregulation of the AGR2 dimerization process is positively correlated with the severity of both diseases [10]. Additionally, AGR2 dimerization is also required for the interaction of AGR2 with other ER-resident proteins, such as heat-shock protein A5 (HSPA5), which in turn induces the activation of the UPR pathway [32].

AGR2 is essential in the production of gel-forming mucins: MUC1 [33], MUC2 [2], MUC5AC and MUC5B [34] in the intestinal epithelial layer, which are essential for the protection of the gastrointestinal tract (GI) against several pathogens, including the majority of viruses and bacteria that cause various human pathologies [35]. In lung tissues, AGR2 has been described to activate the allergen-induced overproduction of mucins during asthma [34]. Furthermore, AGR2 is involved in the stimulation of the wound healing process in the skin by increasing the migration of keratinocytes and the recruitment of fibroblasts [36]. AGR2 has been also shown to regulate angiogenesis by enhancing the activity of vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF2) via direct binding and stimulation of their homodimerization processes [37]. Finally, AGR2 can also play a significant role in metabolism, inducing the expression of lactate dehydrogenase A (LDHA), phosphoglycerate kinase 1 (PGK1), kallikrein 2 (HK2), enolase 1-α (ENO1), as well as glucose uptake and lactate production [38].

Several nuclear, cytosolic, and plasma membrane proteins have been shown to bind AGR2 [39]. For example, under normal conditions, AGR2 has been shown to interact with isoform 1 of uracil-DNA glycosylase (UNG1) protein, which plays a crucial role in activating base-excision repair in mitochondria [40]. Moreover, AGR2 has been also reported to interact with transmembrane emp24 domain-containing protein 2 (TMED2), which can be found in ER and Golgi and functions in vesicular protein trafficking [41].

Widespread interest in AGR2 is however, raised due to its increasingly recognized role in tumor initiation, development, progression, and its resistance to therapy [11, 28]. AGR2 is

overexpressed in different types of solid tumors, including prostate [12], breast [9], lung [10], pancreatic [11], ovarian (8), and oral cancers [7]. A comprehensive summary of the AGR2 expression pattern in various tumor tissues is provided in **Table 1**.

Since the first description of AGR2 expression in human breast cancer in 1998 [42], the extent of AGR2 roles that it plays as an active component of a number of signal transduction pathways has emerged (**Figure 2**).

AGR2 is involved in cellular proliferation and growth through several pathways (**Figure 2**. Cell proliferation and growth; blue color). It targets and regulates Hippo signaling pathway co-activator, yes-associated protein 1 (YAP-1), as well as amphiregulin (AREG), which interacts with epidermal growth factor receptor (EGFR) promoting cellular growth in adenocarcinoma cells [43]. Additionally, AGR2 was identified as an inhibitor of p53 transcriptional response after DNA damage, proving that AGR2 plays a vital role in tumor initiation and progression [44]. AGR2 up-regulates Dual Specificity Phosphatase 10 (DUSP10), which in consequence inhibits p38 mitogen-activated protein kinase (p38 MAPK), preventing the activation of tumor suppressor p53 [45]. Twist Family BHLH Transcription Factor 1 (Twist1) protein increases AGR2 promoter activity via direct binding to the E-box motif, inducing proliferation and growth of breast cancer cells [46]. Finally, a recent study has shown that AGR2 can trigger lung tumor cell proliferation through repressing the tumor suppressor $p21^{CIP1}$ [47].

AGR2 was revealed to be a target of canonical Wnt/β-catenin pathway and is a cancer stem cell marker responsible for enhancing cell proliferation and progression of colorectal cancer [48]. Moreover, the epigenetic regulation of AGR2 by DNA (cytosine-5)-methyltransferase 3A (DNMT3a) was shown to facilitate resistance to 5-azacytidine in colorectal cancer [49]. Additionally, AGR2 was reported to control metformin-dependent activation of AMP-activated protein kinase (AMPK) and silencing of AGR2 induced colorectal cancer sensitivity to chemotherapy [50]. Forkhead box M1 transcription factor (FOXM1) is directly bound to and transcriptionally activates human *AGR2* gene promoter stimulating an invasive phenotype in lung cancer cells *in vivo* [51]. Finally, the glucose metabolism induced by AGR2 can trigger activation of the MUC1/HIF-1α pathway causing progression of endometrial carcinoma [38] (**Figure 2**. Tumor progression and drug resistance; green color).

The cell cycle checkpoint control protein RAD9A was shown to activate the AGR2 expression to promote tumor metastasis and invasion [52]. Recently, AGR2 was established to induce tumor metastasis via regulation of the mTOR Complex 2 (mTORC2) pathway [53]. AGR2 can also promote tumor cell dissemination via post-transcriptional activation of cathepsins B (CTSB) and D (CTSD) [54]. Moreover, AGR2 has been shown to maintain epithelial phenotype by preventing the activation of transforming growth factor-beta (TGF-β) involved in epithelial-mesenchymal transition (EMT) during tumor invasion and metastasis [55]. The knockdown of AGR2 in the metastatic xenograft mouse model reduced tumor growth and metastasis of head and neck squamous cell carcinoma (HNSCC) [56] (**Figure 2**. Invasion and Metastasis; red color).

Knockdown of AGR2 in HNSCC cells caused downregulation of pro-survival markers (Survivin, Bcl2, Bcl2l1, and Cyclin D1), EMT markers (Slug and Snail) and stem cell markers (Nanog, Sox2 and OCT4), suggesting that AGR2 is involved in EMT and self-renewal of cancer stem cells [57] (**Figure 2**. Stemness and cell survival; grey color).

Interestingly, activation of oncogenic Kirsten rat sarcoma (KRAS) mutation $KRAS^{G12D}$ triggered upregulation of the *Agr2* gene, indicating that AGR2 protein is activated downstream of $Kras^{G12D}$ in pancreatic cancer cells [58]. Additionally, mothers against decapentaplegic homolog 4 (SMAD4) was shown to induce AGR2 expression, that in turn up-regulated MUC1 contributing to the development of pancreatic intraepithelial neoplasia (PanIN) and its progression to PDAC [33]. The silencing of AGR2 *in vitro* inhibited ERK/AKT pathway, reduced migration and invasion, and increased apoptosis in pancreatic cancer cells [59]. AGR2 has been also shown to bind and activate Ly6/PLAUR domain-containing protein 3 to stimulate the growth of pancreatic tumors in mice, and blocking of the AGR2-C4.4A pathway reduced pancreatic tumor growth, metastasis, and increased survival of mice in the orthotopic mouse model [60] (**Figure 2**. Pancreatic cancer; yellow color).

Table 1. AGR2 expression in cancer

Ovarian cancer	The elevated levels of AGR2 in plasma are positively correlated to survival in ovarian cancer [8].
Breast cancer	AGR2 expression correlates with poor outcome of patients with ER-positive breast cancer [83].
Cervical cancer	Bioinformatics analysis has revealed that AGR2 is highly expressed in cervical cancer tissue [67], and AGR2 is a potential prognostic factor for this cancer [85].
Endometrial cancer	AGR2 is overexpressed in endometrial cancers and positively associated with high expression of estrogen alpha, progesterone, and androgen receptors [100].
Prostate cancer	AGR2 expression is elevated in prostate cancer [12] and initiates the invasion of prostate cancer cells [76].
Lung cancer	AGR2 is overexpressed in NSCLC [10] and lung adenocarcinoma [101] and is associated with poor survival, especially in younger patients [87]. Immunostaining analysis of 95 NSCLC samples showed elevated AGR2 expression in 66% of the cases [10]. AGR2 expression at the mRNA level is related to lymph nodes metastasis in NSCLC [102].
Nasopharyngeal carcinoma	AGR2 concentration in serum of nasopharyngeal carcinoma (NPC) patients is significantly elevated in comparison to healthy controls. AGR2 serum levels can be used as a marker for the clinical prognosis of NPC [103].
Pancreatic adenocarcinoma	AGR2 was found to be induced in sporadic and familial PanIN lesions, PDAC cells, circulating tumor cells, and metastases. The invasiveness of pancreatic cancer cells correlates with the level of AGR2 expression [54].
Oral cancer	High expression of AGR2 is associated with oral tumor metastasis [7].
Gastric cancer	Elevated expression of AGR2 is related to the progression of gastric cancer and poor survival [104].
Esophageal adenocarcinoma	AGR2 promotes tumor growth of esophageal adenocarcinoma [105].
Cholangiocarcinoma	Upregulation of AGR2 in cholangiocarcinoma promotes cancer cell proliferation, migration, and invasion [106].
Ampullary cancer	AGR2 upregulates proliferation and invasion of ampullary cancer cells [107].
Biliary tract cancer	The AGR2 expression was found to be decreasing with biliary tract cancer progression [108].
Fibrolamellar carcinoma	AGR2 is overexpressed in the majority of fibrolamellar carcinomas [109].
Colorectal cancer	Loss of AGR2 activity is a prognostic factor for colorectal cancer. AGR2 is responsible for the sensitivity of colorectal cancer cells to chemotherapy [110].
Bladder cancer	During bladder cancer, cells secrete AGR2 into urine at higher levels than healthy cells [111].
Papillary thyroid carcinoma	AGR2 is a marker for survival, invasion, and migration of papillary thyroid carcinomas [112].
Head and neck squamous cell carcinoma	AGR2 expression is associated with cancer stem cell and epithelial-mesenchymal transition in high-grade head and neck squamous cell carcinoma [57].
Glioblastoma	The stromal cell-derived factor 1 activates AGR2 to promote EMT progression and the development of glioblastoma [113]. Hypoxia-inducible factor 1 has been shown to regulate AGR2 inducing growth and angiogenesis in glioblastoma [114].
Pituitary adenoma	The serum AGR2 protein levels are significantly elevated in the serum of pituitary adenoma (PA) patients in comparison to healthy controls [70].
Chronic myelogenous leukemia	Upregulation of AGR2 was observed in TKI-resistant chronic myelogenous leukemia (CML) cells [95].

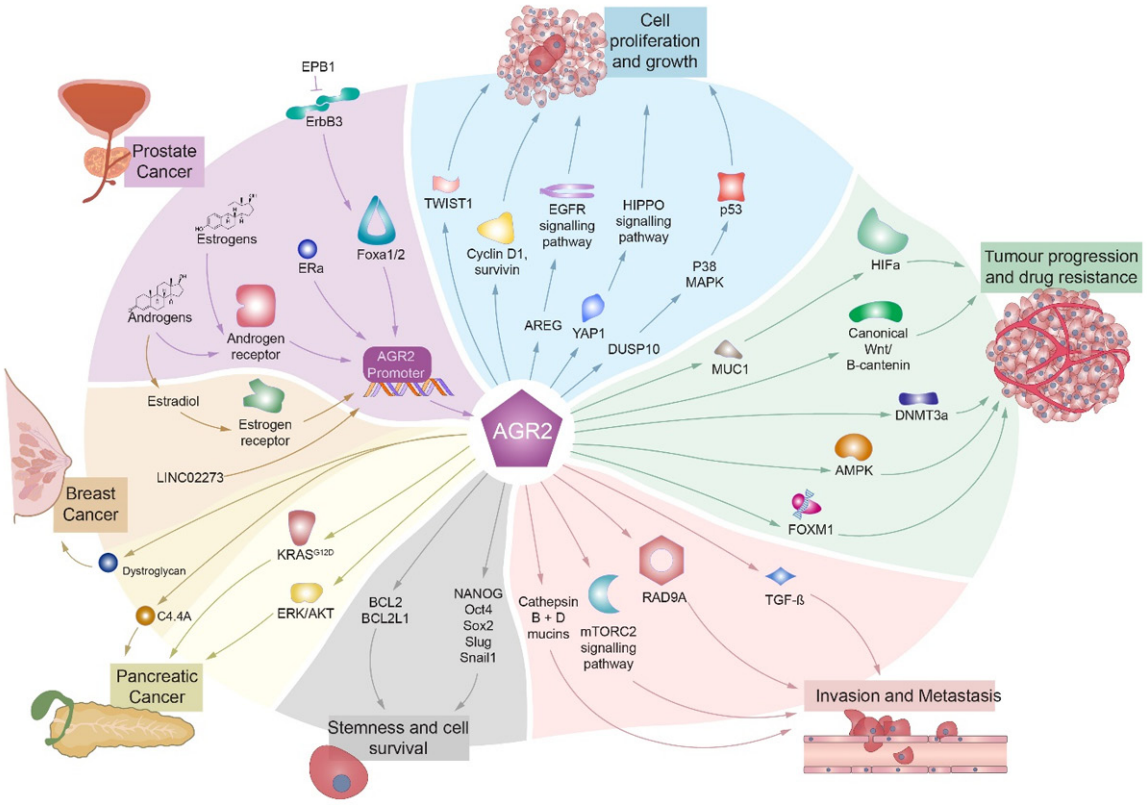

Figure 2. Summary of main AGR2 pathways. Figure shows regulation of cell proliferation and growth (in blue), tumor progression and drug resistance (in green), invasion and metastasis (in red), stemness and cell survival (in grey), pancreatic cancer (in yellow), breast cancer (in orange), prostate cancer (in purple).

AGR2 can be detected in different cellular localizations: intracellular (iAGR2) and extracellular (eAGR2). Interestingly, while healthy cells mainly express iAGR2 in the ER, cancer cells display increased expression of eAGR2 on the cellular surface [54]. The iAGR2 was shown to interact with estrogen receptor alpha (ERα), while eAGR2 induces insulin-like growth factor 1 (IGF-1) signaling via activation of ER-α in breast cancer [61]. Aside from ERα in breast cancer, cyclin D1, pSrc, c-Myc, and survivin were also demonstrated to be expressed downstream of AGR2 [62]. Moreover, AGR2 was shown to interact with C4.4A and dystroglycan (DAG-1) proteins involved in breast cancer metastasis formation through the regulation of estrogen receptor adhesion and functioning [63]. Additionally, AGR2 has been shown to interact with nuclear transcription factor hypoxia-induced factor (HIF)-1α, which has been associated with human breast cancer chemoresistance [64] (**Figure 2**. Breast cancer; orange color).

In prostate cancers, androgens and estrogens have been shown to stimulate the androgen receptor binding to the AGR2 promoter [65]. AGR2 promoter can be also activated by ERα or erb-b2 receptor tyrosine kinase 3 (ErbB3) which enhances Foxa1 and Foxa2. Moreover, ErbB3-binding protein 1 (EBP1) can suppress ErbB3 inhibiting the AGR2 promoter activity in prostate cancer cells [66] (**Figure 2**. Prostate cancer highlighted; purple color).

AGR2 activity is regulated via multiple ways, in addition to already mentioned, several microRNA molecules have been proposed as a potential regulator of AGR2 activity. For example, p53 has been shown to activate miR-3647-5p that in turn suppresses AGR2 expression, inhibiting proliferation and promoting apoptosis of cervical cancer [67], and miR-1291 have been revealed to significantly inhibit AGR2 expression in a pancreatic adenocarcinoma cell line, PANC-1 [68].

Apart from its functional roles in cancer, AGR2 was also established as a promising biomarker for cancer detection as well as a compelling therapeutic target.

AGR2 as a biomarker for cancer detection

AGR2 is attracting considerable interest as a promising biomarker for the detection of the most common cancers due to its elevated expression patterns in premalignant lesions, primary tumors, and metastases. Being a secreted molecule, it can be detected in several biological fluids, including serum, plasma, and urine, being therefore a promising non-invasive biomarker. Importantly, AGR2 was also found to be expressed in circuiting tumor cells (CTCs) in blood specimens of patients with advanced and metastatic cancers and was associated with poor clinical outcome in such patients [69].

The serum AGR2 protein levels were found to be significantly elevated in pituitary adenoma (PA) patients [70], as well as in plasma [8] and serum [71] of ovarian cancer patients. Combined in a panel with cancer antigen 125 (CA125) and midkine (MDK), AGR2 has been evaluated as a potential diagnostic biomarker for symptomatic ovarian cancer patients [72].

AGR2 is also considered a potential biomarker for pancreatic adenocarcinoma (PDAC) detection as it was found at elevated levels in the pancreatic juice of patients with early-stage cancer [73]. Enzyme-linked immunosorbent assay (ELISA) analysis of plasma samples has demonstrated a significant increase of biomarker profile comprising of AGR2, syncollin, olfactomedin-4 (OLFM4), polymeric immunoglobulin receptor (pIgR), and collagen alpha-1(VI) chain in pancreatic cancer patients when assessed against healthy controls [74]. In a patient-derived xenograft model of PDAC, AGR2 is overexpressed in pancreatic cancer stem cells (CSCs), indicating that AGR2 plays a role in PDAC initiation and may be a potential marker of CSCs [75].

Additionally, AGR2 expression has been detected at increased levels in urine sediment of prostate cancer patients [76], suggesting that the development of AGR2 urine ELISA-based test could be useful for the detection of this cancer [77]. Interestingly, AGR2 expression was significantly raised in blood at both messenger RNA (mRNA) and protein level in patients with metastatic prostate cancer [78]. Likewise, immunostaining of prostate cancer has revealed a high expression of AGR2 in bone metastasis [79]. A targeted mass spectrometric assay has been recently developed for quantification of AGR2 in urine and serum specimens [80], which now requires further validation for the early detection of clinically significant prostate cancer in a large cohort study.

The meta-analysis of 20 studies, including 3285 patients, has revealed that increased AGR2 expression is correlated with poor overall survival (OS) of patients with solid tumors, especially with breast cancer [81]. In fact, AGR2 is a well-characterized candidate biomarker for early breast cancer, where it correlates with poor outcome of patients with ER-positive breast cancer [82, 83]. A recent *in vivo* study has shown that the expression of novel long noncoding RNA called LINC02273 is significantly increased in metastatic lesions compared to primary breast tumors. When combined, LINC02273 and AGR2 act as an independent prognostic factor that can be used to predict the OS of breast cancer patients [84]. Moreover, AGR2 has been described as a potential prognostic factor for cervical cancer [85], and the serum AGR2 was shown to be a useful biomarker for early detection, prediction of recurrence, and prognosis of lung adenocarcinomas [86, 87].

Finally, when tested in pre-diagnostic samples, AGR2 combined with CA125, HE4, CHI3L1 and PEBP4 achieved 85.7% sensitivity at 95.4% specificity for detection of ovarian cancer up to 1 year before diagnosis, providing a significant improvement in comparison to CA125 alone [88].

The above studies thus highlight the promising role of AGR2 as both diagnostic and prognostic biomarker, but also suggest that combining AGR2 with other biomarkers and biomarker panels may be a promising strategy for increasing the accuracy of earlier cancer detection. The existence of a number of commercially available anti-AGR2 antibodies provides a first step in the development of ELISAs, lateral flow or other biosensor-type assays that would enable, now necessary, larger scale clinical

validation of promising role of AGR2 as a cancer biomarker.

AGR2 as a therapeutic target

The first antibody developed against AGR2 was the mouse monoclonal antibody, called 184A, which was shown to inhibit the growth of breast cancer cells *in vitro* [89]. Subsequent studies have produced the humanized version of this antibody, 18A4Hu I, and reported that it suppresses the growth of AGR2+ ovarian cancer xenograft [90]. The combinational approach using 18A4 with bevacizumab (targeting VEGF-A) was demonstrated to inhibit tumor growth in an ovarian cancer xenograft mouse model [37]. Recently, the 18A4 antibody was shown to improve survival and prevent AGR2-induced tumor progression by regulating p53 and MAPK pathways, without any toxic effects on major organs in a preclinical lung cancer mice model [91].

We have previously reported for the first time that AGR2 is localized on the surface of PDAC cells. Combined with its expression in both premalignant and malignant cells, as well as in CSC and CTCs in pancreatic cancer, indicated that AGR2 could thus present a promising pantarget for immunotherapy in this malignancy [54]. Recently, we have also demonstrated the successful production of two human-mouse chimeric anti-AGR2 antibodies, P3A5 (IgG2a) and P1G4 (IgG1), that when incubated with human blood, initiated the cellular lysis of eAGR2+ PC3 prostate cancer cells without any damage to iAGR2+ healthy cells, indicating that these antibodies could potentially be implemented for the treatment of several eAGR2 positive solid tumors [92].

The mouse monoclonal antibodies against AGR2 and C4.4A were developed and tested *in vivo* on orthotopic tumors in nude mice models (AsPc-1-Aggressive Cell Model, CaPan-2-Stromal Model and CaPan-2-Regression Study), resulting in significantly reduced tumor growth, metastasis, and increased mouse survival [60]. Moreover, the synthetic single-chain variable fragment monoclonal antibody, scFv4, that can be expressed in mammalian cells has been designed and demonstrated to bind the N-terminal of AGR2 with high affinity [93].

Interestingly, the transduction of dendritic cells with a recombinant adenovirus encoding the *AGR2* gene allowed for AGR2 expression without any significant changes in dendritic cell viability and cytokine secretion. Moreover, it induced the production of AGR2-specific cytotoxic T lymphocytes that were capable of lysing AGR2-expressing colorectal cancer cell lines [94].

While AGR2 is known for its role in solid tumors, due to its interaction with miR-217, AGR2 was also proposed as a potential therapeutic target for the treatment of hematological malignancies, including chronic myelogenous leukemia (CML) [95].

An additional reason for AGR2 targeting is provided by an increasing number of studies that demonstrate the influence of AGR2 on chemotherapy resistance in tumor tissues. For example, AGR2 was shown to enhance the resistance of cells to gemcitabine treatment in pancreatic cancer [96], and it stimulates the development of tamoxifen resistance in breast cancers, proving further that AGR2 is a relevant target for the development of breast cancer therapy [83, 97, 98]. Recently, a study by Cocce et al identified a new targetable pathway for endocrine therapy-resistant breast cancers, based on the transcription factors FOXA1, the membrane receptor LYPD3, and its ligand AGR2. They show that the inhibition of the activity of this pathway using blocking antibodies directed against either LYPD3 or AGR2 inhibits the growth of challenging endocrine therapy-resistant breast cancers in the preclinical model, again providing the rationale for development of humanized antibodies against AGR2 [99].

As the above studies indicate, it is encouraging to see that based on the exceptionally promising properties of AGR2, its favorable localization on cancer cell surface, and the 'holistic' expression in all cancer stages, from stem cells to CTCs and metastases, the conditions are now ripe for its translational development. Two published patents: a US Patent for blocking monoclonal antibodies to AGR2 and its receptor C4.4A (https://patents.justia.com/patent/10428159) and another one for AGR2 blocking antibody (https://patents.google.com/patent/EP2749573A1/en) by Sanofi China, offer a tangible hope of testing of anti-AGR2 antibodies in clinical trials in the near future.

Conclusion

In this review, we have summarized the current knowledge on AGR2 and presented some novel data on its expression in normal human development. Although initially discovered as a developmental gene, AGR2 is generating a considerable amount of interest due to its overexpression in most common cancers, and its important role in tumor initiation, progression, and metastasis. Taken together, this highlights an important role of this protein in both early embryonic development and tumorigenesis. A greater understanding of this relationship will likely continue to contribute to the generation of novel, more effective anti-cancer approaches in humans, both in the field of early detection and in developing novel treatments based on AGR2 targeting.

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Disclosure of conflict of interest

None.

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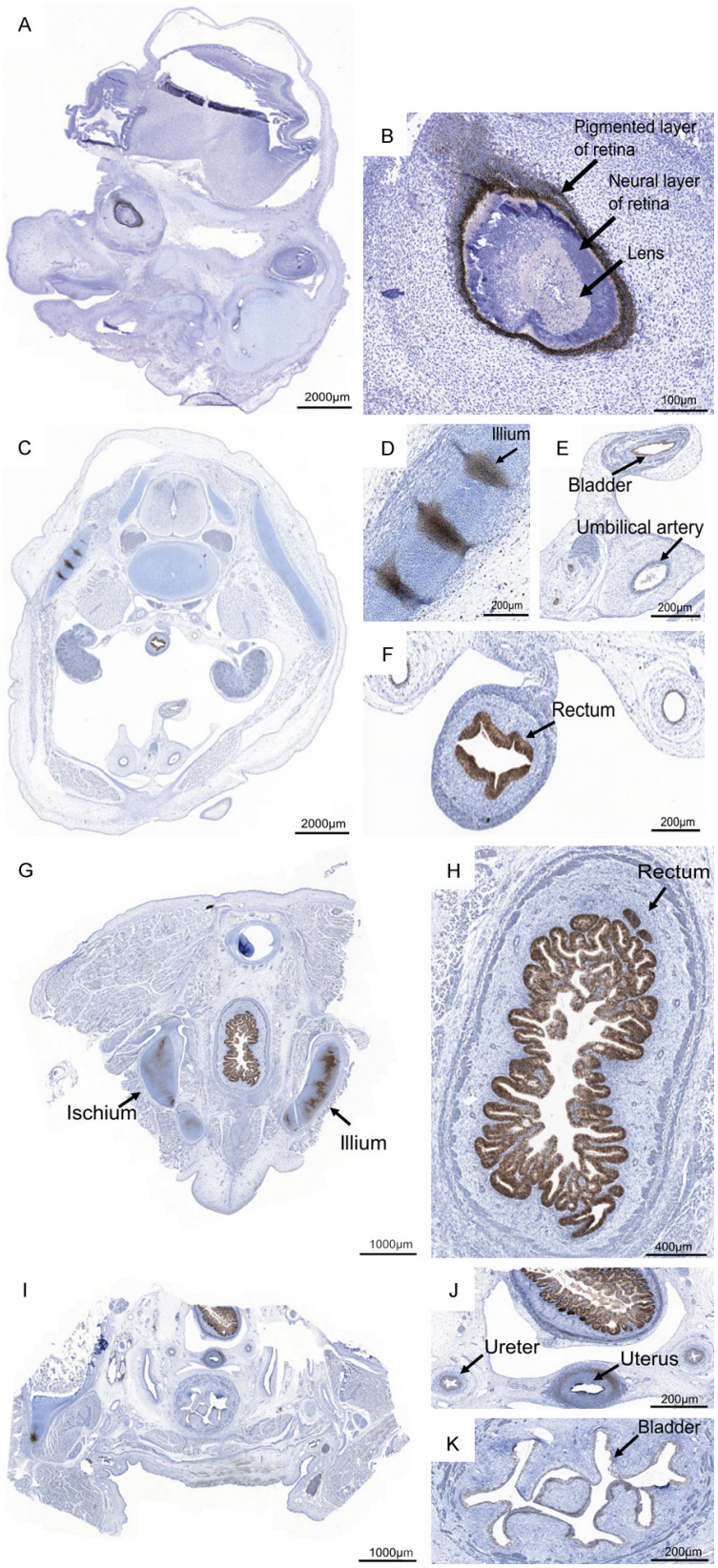

Supplementary Figure 1. AGR2 expression across several tissues. Apart from the gastrointestinal tract (shown in **Figure 1**), AGR2 was also found to be expressed across several other human embryonic tissues including: the pigmented layer of the retina in CS22 (A, B); the ilium, common iliac artery, umbilical arteries, rectum, testis, ureters, and bladder in PCW9 (C-F), ischium, ilium, and rectum in PCW12 (G, H); and uterus, ureter and bladder in PCW14 (I-K).