Aspirin and cancer survival-related pathways: a review and analysis of molecular mechanisms

Manoj Pandey (mpandey66@bhu.ac.in)
Banaras Hindu University
https://orcid.org/0000-0002-1496-9846

Monika Rajput
Banaras Hindu University, Institute of Medical Sciences

Pooja Singh
Institute of Medical Sciences, BHU

Mridula Shukla
Dr. LalPathLabs Ltd

Bin Zhu
National Cancer Institute (NCI), National Institutes of Health (NIH)

Jill Koshial
National Cancer Institute (NCI), National Institutes of Health (NIH)

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Abstract

Background: The benefit of aspirin on cancer survival is debated. Data from randomized clinical trials and cohort studies are discordant, although meta-analysis shows a clear survival advantage when aspirin is added to the standard of care. However, the mechanism by which aspirin improves cancer survival is not clear.

Methods: A PubMed search was carried out to identify articles reporting genes and pathways associated with aspirin and cancer survival. Gene ontology and pathway enrichment analysis was carried out using web-based tools. Gene-gene and protein-protein interactions were evaluated. Crosstalk between pathways was identified and plotted.

Results: Forty-one genes were identified and classified into primary genes (PTGS2 and PTGES2), genes regulating cellular proliferation, interleukin and cytokine genes and DNA repair genes. The network analysis showed a rich gene-gene and protein-protein interaction between these genes and proteins. Pathway enrichment showed the interleukin and cellular transduction pathways as the main pathways involved in aspirin-related survival, in addition to DNA repair, autophagy, extracellular matrix, and apoptosis pathways. Crosstalk of PTGS2 with EGFR, JAK/AKT, TP53, interleukin/TNFα/NFκB, GSK3B/BRCA/PARP, CXCR/MUC1, and WNT/CTNNB pathways was identified.

Conclusions: The results of present study demonstrate that aspirin improves cancer survival by the interplay of 41 genes through a complex mechanism. PTGS2 is the primary target of aspirin and impacts cancer survival through 6 primary pathways: the interleukin pathway, extracellular matrix pathway, signal transduction pathway, apoptosis pathway, autophagy pathway and DNA repair pathway.

Highlights

Evidence before study

There are many studies on cancer prevention using aspirin, and its effect, along with that of other COX2 inhibitors, is well known. However, the effect of aspirin on cancer survival is less studied. Four randomized control trials and other retrospective studies have produced discordant results, potentially due to heterogeneity between the studies and the use of high-dose versus low-dose aspirin. Meta-analysis of the published studies showed a 20% survival benefit for using aspirin in cancer patients when added to the standard of care. The effect is assumedly caused by the inhibition of COX2 receptors. However, the exact mechanism through which this effect is produced is largely unknown.

Added value of the study

To our knowledge, this is the first study to systematically analyze the published literature on the molecular mechanisms of aspirin on cancer survival. After identifying the genes associated with aspirin and cancer survival through a literature search, we applied bioinformatic tools to identify gene-gene
interactions, protein-protein interactions, pathways and the interaction of these pathways and crosstalk between the genes and the pathways. We observed a complex crosstalk between genes and pathways and demonstrated mechanistically how can aspirin affect survival in cancer patients. These findings provide a new impetus for more randomized clinical trials and integration of aspirin with not just chemotherapy, but also with targeted and immunotherapy.

Implications of all available evidence

The evidence to date suggests that adding aspirin to the standard of care in cancer patients can lead to a 30% survival benefit. Aspirin improves cancer survival through a complex mechanism involving the interplay of 41 genes and 6 major pathways (i.e., the interleukin, extracellular matrix, signal transduction, apoptosis, autophagy and DNA repair pathways). The evidence suggests that large and well-designed randomized clinical trials of aspirin plus the standard of care treatment should be conducted along with molecular studies to identify the patients who would best benefit from using aspirin.

Introduction

Despite accumulating evidence of the benefit of aspirin in cancer, its effect on improving cancer survival is still debated since the mechanism by which it impacts cancer survival is not completely understood and the published data are discordant. There have been 4 randomized controlled trials (RCT) showing mixed results from no effect to improved survival. Lipton et al., reported the first randomized trial in 1982 in 66 patients with Duke's B or C, colon and rectal cancers randomized to 600 mg of aspirin or placebo for two years and demonstrated no difference in disease-free or overall survival. A second RCT in 1991 randomized patients with renal cancer to interferon and interferon plus 600 mg of aspirin and reported better results with interferon alone. The third randomized study conducted in 1993 randomized patients with lung cancer to chemotherapy or chemotherapy with aspirin. In this trial, a daily dose of 1000mg of aspirin was used because this dose is supposed to influence platelet function. However, this study also failed to show any benefit of adding aspirin to the standard of care. All these three trials used a relatively high dose of aspirin, ranging from 600mg to 1000mg. However, in 2009, Liu et al., for the first time, reported improved survival in esophageal cancer patients randomized to very low dose of aspirin (50mg). Since then several retrospective and observational studies have reported a survival advantage of adding aspirin to the treatment for various cancers. A meta-analysis of 118 studies, 63 of them specifically reporting on cancer mortality and the rest on all-cause mortality, found a 21% reduction in cancer deaths and about 20% reduction in all-cause mortality (pooled hazard ratio (HR): 0.79; 95% confidence intervals: 0.73, 0.84).

All the studies reported increased instances of bleeding in patients taking aspirin. However, this bleeding was not fatal in any of the cases. It has also been observed that the benefit appears in all cancers and is not limited to any cancer in particular. For example, aspirin use has been associated with improved survival for colorectal cancer (HR = 0.38; 95% CI = 0.17-0.87; p = 0.02), biliary tract cancer, breast
Various mechanisms have been proposed to explain the protective role of aspirin in cancer. Inhibition of COX2, regulating apoptosis and reducing angiogenesis through the prostaglandin pathway is predominant; however, aspirin is also said to improve survival by regulating platelet function and reducing metastasis. Other proposed mechanisms include inhibition of peroxisome proliferator–activated receptor (PARP) and hence interference with the homologous recombinant (HR) DNA repair pathway, nuclear factor-kB (NFκB), and PI3KC pathway regulation. However, no bioinformatic studies have evaluated the interaction of genes associated with cancer survival and their protein products to investigate the possible mechanism by which aspirin can affect cancer survival. Therefore, we conducted this systematic review and bioinformatic analysis to explore the possible mechanism(s) by which the addition of aspirin may produce a survival benefit in cancer patients.

**Material And Methods**

A PubMed search was carried out filtering for English-language papers and using the string ("aspirin" [MeSH Terms] OR "aspirin"[All Fields] OR "aspirins"[All Fields] OR "aspirin s"[All Fields] AND ("cancer s"[All Fields] OR "neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "cancer"[All Fields] OR "cancers"[All Fields]) AND ("mortality"[MeSH Subheading] OR "mortality"[All Fields] OR "survival"[All Fields] OR "survivability"[All Fields] OR "survivable"[All Fields] OR "survivals"[All Fields] OR "survive"[All Fields] OR "survived"[All Fields] OR "survives"[All Fields] OR "surviving"[All Fields]) AND ("genes"[MeSH Terms] OR "genes"[All Fields] OR "gene"[All Fields] OR ("gene s"[All Fields] OR "genes"[MeSH Terms] OR "genes"[All Fields]) OR ("genome"[MeSH Terms] OR "genome"[All Fields] OR "genomes"[All Fields] OR "genome s"[All Fields] OR "genomically"[All Fields] OR "genomics"[MeSH Terms] OR "genomics"[All Fields] OR "genomic"[All Fields])) to identify articles reporting on genes and pathways associated with aspirin and cancer survival. Studies reporting on the molecular mechanism either in clinical or experimental studies on cell lines were evaluated. The search was restricted to English. Non-English studies and studies reporting on NSAID’s other than aspirin or aspirin in combination with other drugs were excluded. The genes were identified, and bioinformatic analysis was performed.

WEB-based Gene Set Analysis Toolkit (Webgestalt) (http://www.webgestalt.org/) was used to perform gene ontology (GO) and pathway enrichment analysis, and GeneMANIA (https://genemania.org) was used for gene-gene interactions. A protein-protein interaction network (PPI) was constructed using NetworkAnalyst (http://www.networkanalyst.ca.), and interacting genes were searched using Search Tool for the Retrieval of Interacting Genes (STRING) (http://string-db.org/). Pathways were created using Reactome (https://reactome.org/PathwayBrowser/), and the rest of the data were manually curated using public databases. Crosstalk between enriched pathways was built using the ShinyGO v0.741 tool (http://bioinformatics.sdstate.edu/go74/). Based on a false discovery rate (FDR) p value of <0.05, the top 30 pathways were enriched. Using the p values from these 30 pathways, a hierarchical clustering tree was constructed. Crosstalk between the genes was prepared manually from the network.
Results

The PubMed search resulted in 189 articles, of which 13 clinical and 32 cell line articles were included to identify genes associated with aspirin and cancer survival. Forty-one genes were identified and were grouped into four categories based on their function (Table 1). The first group includes primary genes controlled directly by aspirin, i.e., \textit{PTGS2} and \textit{PTGES2}. The second group consists of genes involved in cell signaling and cell proliferation like \textit{cMyc}, \textit{EGFR}, \textit{BCL2}, \textit{WNT}, \textit{KRAS}, \textit{WNT6}, \textit{BRAF}, \textit{MUC1}, \textit{PIK3CA}, \textit{PARP1}, \textit{PARP2}, \textit{STAT3}, \textit{MAPK}, \textit{JAK/STAT}, \textit{BAX} \textsuperscript{6,14-32}; the third group, genes for cytokines or their receptors (e.g., \textit{IFN}\gamma, \textit{IL1\beta}, \textit{IL2}, \textit{IL4}, \textit{IL5}, \textit{IL6}, \textit{IL7}, \textit{IL8}, \textit{IL10}, \textit{IL12} (p70), \textit{IL13}, \textit{IL17}, \textit{CXCR1}, \textit{CXCR2}, \textit{PTGS2}, \textit{PTGES2}, \textit{NFKB1}, and \textit{TNFa}) \textsuperscript{30,33-42}; and the fourth group, tumor suppressor genes including \textit{P53}, \textit{BRCA1}, \textit{hMLH1}, \textit{hMSH2}, \textit{hMSH6}, \textit{hPMS2} \textsuperscript{44,45} (Table 1).

Of these 41 genes, 11 (\textit{PTGS2}, \textit{PIK3CA}, \textit{PARP1}, \textit{PARP2}, \textit{VEGFA}, \textit{KDR}, \textit{PTGES2}, \textit{NFKB1}, \textit{P53}, \textit{FLT1}, \textit{VEGFR}) had mechanisms that were directly regulated by aspirin or interacted with aspirin (Figure 1, additional file 1). The remaining 30 genes had indirect involvement or acted through regulation of one of these 11 genes or inflammatory pathways. Focusing on the 11 genes with direct aspirin regulation or interaction formed the basis of the main analysis. The inflammatory pathway was used for secondary analysis after categorizing the genes in the above four groups, GeneMANIA identified 34 co-expression, 2 genetic, 54 physical and 23 predicted interactions between these 11 genes (Figure 1a, additional file 1). A total of 44 shared protein domains were identified among the interacting genes. Protein-protein interactions occurred in 3 clusters of 3, 5 and 2 proteins (Figure 1b). \textit{PTGES2} interacted with only \textit{PTGS2}. All other proteins interacted with each other except \textit{PARP2}, which had no interaction with the \textit{FLT} protein. This analysis confirmed that \textit{PTGS2} and \textit{PTGES2} are the central genes through which aspirin impacts cancer survival.

Given this confirmation that \textit{PTGS2} and \textit{PTGES2} are the primary genes inhibited by aspirin, we evaluated how these two primary genes interact with the other 39 genes, which we categorized into three groups based on their function, i.e., cell signaling and cell proliferation, cytokines and interleukins and tumor suppressor genes. The analysis of \textit{PTGS2} and \textit{PTGES2} with genes participating in cell signaling and proliferation showed 37\% co-expression and 20\% physical interaction with 18 primary nodes and 20 secondary nodes reflecting how these genes relate to one another (figure 2a). The protein-protein network of these three groups of genes had 18 nodes and 62 edges (figure 2b). The \textit{p} value of network was highly significant (\textit{p}=2.75e\textsuperscript{-15}), suggesting significantly more interactions than expected. The highest number of interactions with \textit{PTGS2} were seen with the \textit{MAP} kinase and \textit{EGFR} pathways, suggesting that COX2 (PTGS2) suppression may lead to inhibition of MAP kinase and EGFR pathways and hence reduction of cellular growth and proliferation.

We then examined the interaction of \textit{PTGS2} and \textit{PTGES2} with the interleukin and cytokine genes. The gene-gene network showed a high level of co-expression of these genes (85\%), which was expected as the \textit{COX2} gene and prostaglandin control the synthesis of interleukins and cytokines (additional figure 1a). The protein-protein network of these genes too was equally rich with 18 nodes and 122 edges.
The PPI enrichment value was highly statistically significant \((p=1.0 \times 10^{-16})\), indicating that there were many more interactions than expected by chance.

The network of \(PTGS2\) and \(PTGES2\) with tumor suppressor genes showed nearly 70% physical interactions with 21 secondary nodes between these genes (additional figure 2a). While the protein-protein interaction network (additional figure 2b) had 5 nodes and 5 edges, the interaction was not statistically significant \((p=0.07)\), suggesting that COX2 does not directly regulate these tumor suppressor genes. In contrast, the cell signaling and proliferation proteins (additional figure 2c) and the cytokine and interleukin gene proteins (additional figure 2d) did interact with the tumor suppressor gene proteins \((p<1.0 \times 10^{-16})\), suggesting that aspirin indirectly regulates tumor suppressor genes through its impact on cell signaling and proliferation gene and interleukin and cytokine gene expression. All gene-gene interactions between the four groups (primary genes, cell signaling and cell proliferation genes, cytokine and interleukin genes and tumor suppressor genes) are detailed in additional file 2.

Pathway analysis with Reactome showed the largest number of genes interacted with interleukin (additional file 3) and signal transduction pathways (additional file 4). Additional interactions were identified in DNA repair, autophagy, extracellular matrix, and apoptosis pathways (additional file 5-8). Based on these pathways, a pathway diagram was created that incorporated most genes and pathways (figure 3a, 3b). Crosstalk between the genes and the pathways is shown in figure 4.

**Discussion**

There are many studies on cancer prevention using aspirin, and its effect, along with that of other COX2 inhibitors, is well known. The results of the randomized controlled trials have been mixed, the findings from the low-dose trial and subsequent nonrandomized observational studies suggest that the addition of aspirin to the standard of care improves cancer survival. However, the mechanism by which aspirin improves survival is not clear. This study is the first to our knowledge to comprehensively evaluate the literature and apply bioinformatics to investigate the underlying mechanism.

Our literature search identified 41 genes related to aspirin and cancer survival that fell into four clusters including the primary genes directly regulated by \((PTGS2\) and \(PTGES2\)), oncogenes and cell cycle regulators (16 genes), interleukin and cytokines regulators (16 genes) and tumor suppressor genes (7 genes). Of 11 main genes that are regulated by aspirin or interact with aspirin, the DNA repair pathway, especially the homologous recombination (HR) pathway, showed co-expression of \(PTGS2\) with \(TP53\), \(PARP1\) and \(PARP2\).

Several previous studies have shown that aspirin can affect DNA repair genes. For example, treatment of a DNA MMR competent/p53 mutant colorectal cancer cell line with aspirin for 48 hours led to DNA damage pathway gene expression, including \(BRCA1\).\(^{45}\) A later study found that feeding aspirin to Dalton cell lymphoma bearing mice, resulted in cell cycle arrest in G0/G1 phase.\(^{34}\) In addition, aspirin was found to lower the number of somatic mutations, including mutations in \(TP53\) in Barret’s esophagus patients.\(^{40}\)
The potential of altering the levels of NFκB and its use as a biomarker is being tested in ASAMET (NCT03047837) trial, wherein both aspirin and metformin are given to patients with stage I-III colorectal patients, and the results are awaited.\textsuperscript{46}

Despite the availability of cell-line and animal model data, it is still not clear why there is co-expression of $COX2$ ($PTGS2$) with DNA repair pathway genes. Rationally as COX2 increases cellular proliferation, it should inhibit DNA repair and apoptosis proteins, as is seen with counter regulation of the BCL2/BAX/Caspase cascade. Two possible mechanisms are through the regulation of $MYC$ and $MUC1$. Alternatively, the co-expression of COX2 and DNA repair pathway genes could be due to an increase in DNA synthesis, thereby increasing the DNA repair cascade to check the DNA that is being newly synthesized. We demonstrate regulation of DNA repair genes through crosstalk of the interleukin pathway that regulates $JAK1$, $PI3KC$ and $AKT$, which in turn regulate the p53 pathway, and another interaction through CXCR regulation through Muc1 (figure 4). This hypothesis of complex crosstalk might be more plausible, but the exact mechanisms involved are not clearly understood. Additional studies are required to understand this process fully.

The effect of $PTGS2/PTGES2$ on interleukin pathway activation is more obvious and can be explained by its effect on arachidonic acid metabolism and increased synthesis of interleukins and cytokines. Similarly, the effects on cellular proliferation and signal transduction pathways are also explainable through direct\textsuperscript{45} or interleukin mechanisms.\textsuperscript{23,33,34,47}

Though most of the RCTs failed to demonstrate any survival benefit of the addition of aspirin to the standard of care, the benefit is seen in one RCT of low-dose aspirin, in observational studies and in a meta-analysis that included these observational studies. This inconsistency could be due to use of different doses of aspirin or inherent biases in the observational studies like adherence bias, healthy user bias, etc. Further, most earlier studies were based on the effect of aspirin on the blockade of the COX2 or thromboxane (Tx) pathway and assumed this blockade to be the main mechanism through which aspirin exerts its benefit on cancer survival. The present study is the first to demonstrates that aspirin can improve survival by an interplay of 41 genes, two of which ($PTGS2$ (COX2) and $PTGES2$) are the primary target of aspirin. The effect is mediated through 6 primary pathways: the interleukin, extracellular matrix, signal transduction, apoptosis, autophagy and DNA repair pathways. This study demonstrates that aspirin's regulation of cancer is more complex than previously understood. More randomized controlled trials evaluating the benefit of adding aspirin on cancer survival and incorporating molecular studies assessing interleukins and cytokines are required to fully understand the mechanisms by which aspirin appears to improve survival in cancer patients.

**Declarations**

**Authors contributions**

MP: Concept and design, data interpretation, editing of the manuscript
MR: data mining, text mining, graphics, bioinformatic analysis, interpretation, draft manuscript preparation

PS: Literature search, draft manuscript preparation, organizing the text, tables and graphics

MS: Pathological interpretation of carcinogenic and protective pathways, manuscript editing

BZ: Analysis and interpretation, editing of the manuscript

JK: Concept and design, pathway interpretation and manuscript editing

All authors have read and approved the final manuscript

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**Conflicts of interests**

The authors declare that there are no conflict of interests.

**Ethics and consent**

As the study involves review of published data, text and data mining and bioinformatics, the ethical approval and consent are not applicable.

**Data availability**

All the data are submitted as additional files or included in the manuscript.

**Acknowledgement**

None

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https://doi.org/10.1186/s12885-018-5126-7

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### Tables

**Table 1:** The genes identified as effector to increase survival in patients receiving Aspirin, their grouping according to the function
<table>
<thead>
<tr>
<th>Group</th>
<th>Gene</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 primary genes</td>
<td>Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase) PTGS2/COX2</td>
<td>Synthesis of enzyme cyclooxygenase 2 (Cox 2) that convert arachidonic acid to prostaglandin endoperoxide H2</td>
</tr>
<tr>
<td></td>
<td>PTGES2 - prostaglandin E synthase 2</td>
<td>Encode membrane-associated prostaglandin E synthase, that catalyzes the conversion of prostaglandin H2 to prostaglandin E2, also activate gamma-interferon-activated transcription element (GATE)</td>
</tr>
<tr>
<td>2 Oncogenes and cell cycle regulators</td>
<td>MYC- MYC proto-oncogene, bHLH transcription factor</td>
<td>encodes a nuclear phosphoprotein that plays a role in cell cycle progression, apoptosis and cellular transformation</td>
</tr>
<tr>
<td></td>
<td>EGFR- epidermal growth factor receptor</td>
<td>Encode for epidermal growth factor receptor protein that regulate epithelial tissue development and homeostasis</td>
</tr>
<tr>
<td></td>
<td>BCL2-B cell lymphoma 2</td>
<td>Encode protein that regulate apoptosis. Increase cellular survival by inhibiting pro apoptotic proteins</td>
</tr>
<tr>
<td></td>
<td>WNT1-WNT family member 1; Proto-oncogene Int-1 homolog</td>
<td>encode secreted signaling proteins that increase cell growth and division</td>
</tr>
<tr>
<td></td>
<td>KRAS- Kirsten rat sarcoma viral oncogene homolog</td>
<td>Encode tumor oncogene Kras protein part of RAS/MAPK pathway among other signal transduction pathway.</td>
</tr>
<tr>
<td></td>
<td>WNT6- Wingless-type MMTV integration site family, member 6</td>
<td>Highly conserved gene encode wnt6 protein that regulate cell growth and differentiation through wnt pathway</td>
</tr>
<tr>
<td></td>
<td>BRAF- raf murine sarcoma viral oncogene homolog B</td>
<td>Member of raf kinase family, controls cell proliferation by regulating signal transduction protein kinases</td>
</tr>
<tr>
<td></td>
<td>MUC1- Mucin 1, cell surface associated; polymorphic epithelial mucin (PEM); epithelial membrane antigen (EMA)</td>
<td>Protective function as binds to foreign pathogens, also regulates cell signalling</td>
</tr>
<tr>
<td></td>
<td>PIK3CA- Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha</td>
<td>Encode oncogenic protein that catylyze ATP to phosphorylate PtdIns, PtdIns4P and PtdIns(4,5)P2.</td>
</tr>
<tr>
<td></td>
<td>PARP1- Poly [ADP-ribose] polymerase 1 (PARP-1); NAD+ ADP-ribosyltransferase 1 or poly[ADP-ribose] synthase 1</td>
<td>ADP-ribosylation, repair of single strands break, and with BRCA double stranded breaks</td>
</tr>
<tr>
<td></td>
<td>PARP2- Poly [ADP-ribose] polymerase 2</td>
<td>encodes poly(ADP-ribosyl)transferase-like 2 protein, a catalytic domain capable of catalyzing a poly(ADP-ribosyl)ation reaction</td>
</tr>
<tr>
<td></td>
<td>STAT3- Signal transducer and activator of transcription 3</td>
<td>Transcription activator after being phosphorylated by receptor-associated Janus kinases (JAK)</td>
</tr>
<tr>
<td></td>
<td>JAK1- Janus kinase 1</td>
<td>tyrosine kinase protein essential for signaling type I and type II cytokines.</td>
</tr>
<tr>
<td><strong>Group 3 – cytokines and Interleukins</strong></td>
<td><strong>IFNG- Interferon gamma</strong></td>
<td><strong>IL1B- Interleukin 1Beta also known as leukocytic pyrogen, leukocytic endogenous mediator, mononuclear cell factor, lymphocyte activating factor</strong></td>
</tr>
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<td>-----------------------------------------</td>
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</tbody>
</table>
| MAPK1- Mitogen-activated protein kinase 1, also known as ERK2 | Promote cell division | member of the MAP kinase family regulating extracellular signal-regulated kinases | STAT- Signal transducer and activator of transcription | intracellular transcription factors that mediate cellular immunity, proliferation, apoptosis and differentiation | BAX- bcl-2-like protein 4 | Forms heterodimers with BCL2 and regulates apoptosis. It’s a proapoptotic factor stimulate release of caspases. | Group 3 – cytokines and Interleukins | Class II interferon, activator of macrophages and inducer of major histocompatibility complex class II molecule expression | IL1B- Interleukin 1Beta also known as leukocytic pyrogen, leukocytic endogenous mediator, mononuclear cell factor, lymphocyte activating factor | Interleukin 1 family of cytokines, released from macrophages, activated by caspase it mediates inflammatory response, and other cellular activities, including cell proliferation, differentiation, and apoptosis | IL2- Interleukin 2 | Improves tolerance and immunity, primarily via its direct effects on T cells. Improves cell killing by NK cells and activated T cells | IL4- Interleukin 4 | induces differentiation of naive helper T cells (Th0 cells) to Th2 cells | IL5- Interleukin 5 | stimulates B cell growth and increases immunoglobulin (IgA) secretion | IL6- Interleukin 6 | pro-inflammatory cytokine secreted by macrophage and an anti-inflammatory myokine. It binds to pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) | IL7-Interleukin 7 | hematopoietic growth factor secreted by stromal cells in the bone marrow and thymus. Participate in proliferation during certain stages of B-cell maturation, T and NK cell survival, development and homeostasis | IL8- Interleukin 8 | chemokine produced by macrophages and epithelial cells. Induces chemotaxis, and angiogenesis | IL10- Interleukin10; human cytokine synthesis inhibitory factor (CSIF) | Primarily produced by monocytes, participate in immunoregulation and inflammation. Blocks NF-κB, regulate JAK-STAT pathway | IL12B- Interleukin 12 subunit beta; (natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor p40, or interleukin-12 subunit p40) | Secreted by activated macrophages, inducer of Th1 cells development. | IL13- Interleukin 13 | Functions similar to IL3. induces a class of protein-degrading enzymes, known as matrix metalloproteinases (MMPs) | IL17- interleukin 17 | immune regulatory cytokine, induce and
mediate proinflammatory response. Induce expression of keratinocytes

<table>
<thead>
<tr>
<th>CXCR1 - C-X-C motif chemokine receptor 1; Interleukin 8 receptor, alpha or CD181</th>
<th>G-protein-coupled receptor family binds to IL8 and increases cellular proliferation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXCR2 - C-X-C motif chemokine receptor 2; Interleukin 8 receptor, beta</td>
<td>binds with IL8, and transduces the signal through a G-protein-activated second messenger system. Mediate angiogenic effect of IL8 on endothelial cells</td>
</tr>
<tr>
<td>NFkB - Nuclear factor kappa-light-chain-enhancer of activated B cells</td>
<td>A primary transcription factor and regulator of signalling response, suppress TNF induce cytotoxicity, regulate TRAF1 and TRAF2</td>
</tr>
<tr>
<td>TNF - Tumor necrosis factor ( cachexin, or cachectin; also called as tumor necrosis factor alpha or TNF-α)</td>
<td>adipokine and a cytokine, binds to two receptors TNFR1 and 2. Regulates NFkB, TRAF1 and 2, activate MAP kinase pathway, cell differentiation and proliferation</td>
</tr>
<tr>
<td>4- Tumor suppressor genes</td>
<td></td>
</tr>
<tr>
<td>TP53 - tumor protein 53; transformation-related protein 53 (TRP53)</td>
<td>Tumor suppressor gene, DNA repair, Cell cycle arrest in G1/S phase, initiator of apoptosis, senescence response to short telomeres</td>
</tr>
<tr>
<td>BRCA1 - Breast cancer type 1 susceptibility gene</td>
<td>Repair of double stranded DNA breaks, mismatch repair,</td>
</tr>
<tr>
<td>BRCA2 - breast cancer type 2 susceptibility gene</td>
<td>Encodes BRCA2 protein with functions similar to that of BRCA1. Form BRCA1-PALB2-BRCA2 complex.</td>
</tr>
<tr>
<td>MLH1 - mutL homolog 1</td>
<td>DNA mismatch repair genes,</td>
</tr>
<tr>
<td>PMS2 - PMS1 homolog 2</td>
<td>DNA mismatch repair genes</td>
</tr>
<tr>
<td>MSH2 - mutS homolog 2</td>
<td>Microsatellite instability associated gene altered in microsatellite sequences (RER+ phenotype) in HNPCC</td>
</tr>
<tr>
<td>MSH6 - mutS homolog 6</td>
<td>DNA mismatch repair, forms MSH recognition complex with MSH2</td>
</tr>
</tbody>
</table>

**Figures**
Figure 1

A) Gene-gene interaction of the 11 main genes that explain how aspirin can improves cancer survival (grey shaded nodes are primary input genes, grey non-shaded nodes are the secondary genes showing interaction with primary genes) B). Protein-protein interaction of 11 proteins synthesized by the 11 main genes identified showing three clusters of proteins; the first cluster is co-expression of PTGS2, PTGES2 and P53 (red), while the second cluster is of cell cycle regulators (green) and the third is of DNA repair genes (blue)

Figure 2

Figure 3

A) Pathway enrichment with crosstalk between identified pathways B) Tree analysis of the pathway based on the $p$ value for each pathway on enrichment. Pathways with many shared genes are clustered together. Bigger dots indicate more significant $P$-values.
Figure 4

Proposed mechanism of aspirin in inhibiting cellular proliferation and increasing in autophagy, DNA repair and apoptosis through complex crosstalk. Genes in red are the primary genes identified through data mining.

Supplementary Files

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• additionalfile4signaltransductionpathwayandourgenes.pdf
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• additinalfile6ECMpathwaywithidentifiedgenes.pdf
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