Human Interferon Alpha-2b: A Therapeutic Protein for Cancer Treatment

1. Introduction

Cancer is a first cause of death in developed countries and the second cause of death in developing countries. It was reported that approximately 12.7 million cancer cases occur worldwide with 7.6 million of mortality rate. About 64% of deaths arise in developing countries. Breast cancer in women and lung cancer in men are the biggest cause of death, followed by stomach, liver, cervix, and prostate cancers. Cancer is a disease caused by uncontrolled cell growth. Growth of these cells causes damage to surrounding tissues and spreads to other tissues via the blood or lymphatic circulation [1, 2].

Cancer is generally derived from one cell with accumulated changes in several different genes. Mutation can be caused by exposure to carcinogens, radiation, or infection by bacteria and viruses. Accumulation of mutations causing abnormal cell growth is known as premalignancy. Genes that are involved in cancer are divided into two categories: oncogene and tumor suppressor. Protooncogenes are protein-coding genes that promote cell division, including growth factors (e.g., platelet-derived growth factor, epidermal growth factor, and fibroblast growth factor), growth factor receptors (e.g., HER2 ERBB2, ERBB1, and IGF-1R), and proteins in signal transduction pathways (e.g., RAS, ABL, and RAF). Oncogenes are the mutated forms of protooncogenes.

Mutation in oncogenes causes different characteristics or overexpression of protein that triggers the growth of abnormal cells. Tumor suppressor genes are protein-coding genes that suppress cell division, such as RBL and p53. Mutations in tumor suppressor lead to loss of suppressor activity so that the cells become abnormal in growth. Abnormal cells are used as a therapeutic targets for cancer treatment [2, 3].

Cancer treatment can be performed in various ways, such as surgery, chemotherapy, radiotherapy, immunotherapy, gene therapy, or protein therapy. Recombinant therapeutic proteins that have been widely used in cancer are enzymes (Elspar, Oncaspar, and Elitek), toxins (denileukin difitox or ontak), monoclonal antibodies (Zevalin, Mylotarg, Bexxar, Herceptin, Avastin, Erbitux, Rituxan, Vectibix, and Campath), and cytokines (interleukin-2, interferon-a3, interferon-β1, and interferon-α2b). rhIFNα-2b was first approved as therapeutic protein by United States Food and Drug Administration (FDA) in 1986. It is used for the treatment of hairy cell leukemia and currently as much as 86 countries have been using rhIFNα-2b in hepatitis and cancer treatments [1, 4]. rhIFNα-2b is widely applied in monotherapy or in combination therapy with other drugs. rhIFNα2b is combined with ribavirin, lamivudine, or adeovir on hepatitis treatment and combined with cytarabin,
vinblastine, 5-fluorouracil, tamoxifen, or interleukin-2 on cancer treatment [4–6].

2. Molecular Characteristics of hIFNα-2b

The interferons (IFNs) were first introduced in 1957 as antiviral molecules. Based on their receptor types on the cell membrane surface, IFNs are classified into type I and type II. Type I consists of IFNα, IFNβ, IFNω, and IFNτ, while type II consists of IFNγ. IFN type I is a family of cytokines in which amino acid sequence similarity reaches 30–80%. Receptors that are recognized by the type I are grouped into two subunits, IFNAR-1 and IFNAR-2. IFNAR-2 consists of three types, namely, IFNAR-2a, IFNAR-2b, and IFNAR-2c, respectively. IFNAR-2c has an important role in ligand binding and signal transduction, whereas IFNAR-2a and IFNAR-2b are competitive inhibitors that prevent IFN from binding to IFNAR-2c. IFNα has wide biological activities ranging as antiproliferation, immunomodulation, and antiviral. IFNα coding genes are located on human chromosome number 9. They have no introns and encode polypeptide chain of 165-166 amino acids. Some IFNαs are unglycosylated but some are glycosylated proteins with different degrees of glycosylation [7–9]. hIFNα-2b molecule is a glycoprotein consisting of 166 amino acids with O-glycosylated threonine at position 106. Two disulfide bonds are formed by cysteines at position 1 and 98 as well as 29 and 138 (Figure 1). Bond formed by positions 1 and 98 is not required in biological activity. Amino acid residues that are important in the biological activity are Leu30, Lys31, Arg33, His34, Phe36, Arg120, Lys121, Glu132, and Glu146. Based on crystal structure that is mediated by zinc dimer, each monomer of rhIFNα-2b consists of five alpha helices (called helix A to E) that are connected by a loop AB, BC, CD, and DE. Residues that are important in the structural integrity are Phe36, Tyr122, and Tyr129. Residues that are important in receptor binding are the AB loop (Arg22, Leu26, Phe27, Leu30, Lys31, Arg33, and His34), helix B (Ser68), helix C (Thr79, Lys83, Tyr85, and Tyr89), D helix (Arg120, Lys121, Gln124, Lys131, and Glu132), and helix E (Arg144 and Glu146). Figure 1 shows a structure of rhIFNα-2b [4, 10, II].

3. Molecular Mechanism of hIFNα-2b as Anticancer

Antiproliferative activity of hIFN-α consists of direct and indirect activities. Direct activity occurs through cancer cell growth inhibition by cell cycle arrest, apoptosis, or differentiation. Indirect activity occurs through activation of immune cells such as T cells and natural killer cells, inhibition of vascularization (angiogenesis), and induction of cytokines. The antiproliferative activity is the result of gene expression regulation. It is initiated by signal transduction pathways and transcriptional activation of JAK-STAT. Study of gene expression in melanoma cell lines (WM9), fibrosarcoma (HT1080), embryonic fibroblasts, and human dendritic cells reported that hIFNαs regulate more than 300 genes of signal transduction pathways in cells [8, 12].

![Figure 1: Molecular structure of hIFNα2b (by PyMOL).](image-url)
MEK ERK pathway inhibition by hIFNα2b has been widely reported. In experiments, using CD4 + T cells stimulated by anti-CD3 and interleukin-2 (IL-2), it was observed that hIFNα2b can prevent the G0/G1 phase of the cell cycle from entering S phase. As a result, cells cannot perform DNA replication and proliferation does not occur. Several publications reported that the inhibition of proliferation occurred because hIFNα2b may induce enzymes that can inactivate phosphatases PP2A and regulate docking protein that inhibits the interaction of ERK with MEK or MEK interaction with other kinases. Inhibition would decrease the activity of cyclin-dependent protein kinase (CDK-2 and CDK-4) and decreased the expression of cyclin D and E protein as the driving cell division. Inhibition of p21 expression leads to increase p27 Waf1/kip1 (inhibitor of cell division) and decrease phosphorylation of RB/p105 [18, 19].

Mechanism of antiproliferation hIFNα2b occurs not only through regulation of protein synthesis and selective translation of proteins involved in inhibition of proliferation, but also through apoptosis as shown in Figure 3. There are two main pathways of apoptosis in cells that are activated: hIFNα2b family receptor signal transduction through tumor necrosis factor alpha (TNF-α) and the release of cytochrome c by mitochondria. Both of these pathways activate caspase signaling cascade resulting in DNA fragmentation and cell death. Induction of cell death occurs through TNF-α receptor family, namely, TNF-a/TNF-aR, FasL/Fas, Apo1, TRAIL/TRAILR, and Apo2. hIFNα2b contributes to an increase in p53 protein response to stress signaling and activation of p38 that plays a role in cell death. Additionally, hIFNα2b can activate PKR which has a variety of protein substrates such as eukaryotic initiation factor 2 (eIF2), NF-KB, IRF-1, p53, STAT1, and NF-90. These proteins may result in the control of cell division, differentiation, and apoptosis. PKR regulates transcription and translation to produce proteins Fas, p53, and Bax can trigger cell death via caspase pathway [14, 20, 21].

The expression of caspase pathway proteins, such as caspase-8 and caspase-9, caspase-3, caspase-6, and caspase-7, is also regulated by hIFNα2b. Caspase pathway is initiated by DNA damage signaling. The signal will cut BID protein and alter mitochondrial membrane permeability to release cytochrome c. Cytochrome c protein that activates Apaf-1 and caspase-9 will result in cell death. hIFNα2b also increases the protein expression of caspase-3 and caspase-7 as well as caspase-8 protein that produces DNA fragmentation. Induction of apoptosis occurs through TNF-a receptor family, namely, TNF-a/TNF-aR, FasL/Fas, Apo1, TRAIL/TRAILR, and Apo2. hIFNα2b contributes to the increase in p53 protein response to stress signaling and activation of p38 that plays a role in cell death. Additionally, hIFNα2b can activate PKR which has a variety of protein substrates such as eukaryotic initiation factor 2 (eIF2), NF-KB, IRF-1, p53, STAT1, and NF-90. These proteins may result in the control of cell division, differentiation, and apoptosis. PKR regulates transcription and translation to produce proteins Fas, p53, and Bax can trigger cell death via caspase pathway [14, 20, 21].

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4. The Use of hIFNα-2b as Therapeutic Protein for Cancer Treatment

Interferons licensed for antitumor applications are hIFN-α2a (Roferon-A, Hoffmann-La Roche) and hIFN-α2b (Intron A, Schering-Plough). The most oncological indication of hIFN-α2b includes hairy cell leukemia, melanoma, follicular lymphoma, renal cell carcinoma, AIDS-related Kaposi’s sarcoma, and chronic myelogenous leukemia. The clinical applications of rhIFNα-2b can be summarized in Table 1.
4.1. Hairy Cell Leukemia. Hairy cell leukemia is characterized by mononuclear cells of B-lymphocyte origin in the peripheral blood that have prominent cytoplasmic projections staining with tartrate-resistant acid phosphatase. It is also identified by its typical pattern of infiltration in the bone marrow and spleen. hIFN-α2b was first approved for use in hairy cell leukemia in 1986. The route of administration for hairy cell leukemia is subcutaneous and the recommended dose is 2 million U/m² three times weekly for 12 months [22]. The first report of successful story of IFN was in 1984. Seven patients received 3 million U of partially purified (leukocyte) human IFN intramuscularly daily. Three of seven patients achieved a complete remission and four a partial remission [23]. Purified IFNα-2b synthesized by using recombinant DNA technology (Intron A, Schering Corporation) demonstrates similar activity [24]. It was also reported that hIFNα-2b was given to 50 patients. The dose was 2.0 × 10⁶ IU/m² subcutaneously three times weekly. At 24 months, there were 38 patients remaining. During the two years of continuous IFN treatment none of the patients showed any signs of relapse. The IFN therapy was generally well tolerated, but 24 month evaluation showed mild toxicity in about 76% of the patients [25]. There was a study which reported unexpected high incidence of second neoplasm in patients after hIFNα-2b treatments with the same dose for 12 to 18 months of therapy. There were 13 patients from 69 patients (six were hematopoietic origin and the remaining were adenocarcinomas) who developed second neoplasm [26].

4.2. Melanoma. According to the national cancer institute, melanoma is defined as a form of cancer that begins in melanocytes. Melanocytes are cells that make the pigment melanin. Melanoma may begin in a mole (skin melanoma) but can also begin in other pigmented tissues, such as in the eye or in the intestines. The use of high dose of IFN2b for the adjuvant therapy of stage IIB and III melanoma patients was approved by FDA in 1995. A study by Kirkwood et al. [27] in 287 patients compared intravenous administration of hIFNα-2b at 20 MU/m² for 1 month and subcutaneous administration at 10 MU/m² for 48 weeks with observation alone. It was reported that prolongation of disease-free survival and prolongation of overall survival occurred in comparison to observation. In 1989, the Scottish melanoma group applied a randomized trial to compare observation alone with 6 months’ therapy with subcutaneously low dose interferon at 3 MU/day (three times weekly). The result showed that there was a statistically significant improved disease-free survival for up to 24 months [28]. A Systematic Review of Randomized Controlled Trials by Lens and Dawes [29] stated that there was no clear benefit of hIFNα-2b on overall survival in melanoma patients. A large randomized controlled trial is needed to study the effectiveness and beneficiary of hIFNα-2b treatment.
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<td>Prolongation of survival and remission duration. Prolongs survival in patients with advanced follicular lymphoma. The role of interferon as initial and maintenance therapy in patients with newly diagnosed FL did not demonstrate any advantage.</td>
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<td>Renal Cell Carcinoma</td>
<td>Long term of combination hIFNα-2b and interleukin-2 administered subcutaneously in 50 patients. Neprectomy followed by hIFNα-2b administration.</td>
<td>Objective responses was observed in 9 of 50 (18%) patients and 6 of whom (12%) achieved a complete response. Overall median survival is 12 months, six patients were surviving at a median follow-up of 24 months, and three (6%) are still progression-free. Improved survival of 120 metastatic RCC patients.</td>
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4.3. Follicular Lymphoma. National cancer institute defines follicular lymphoma (FL) as a type of B-cell non-Hodgkin lymphoma that is usually indolent. The tumor cells grow as groups to form nodules. A meta-analysis to evaluate the role of hIFNα2 in FL reported prolongation of survival and remission duration when it has been given in the context of relatively intensive initial chemotherapy at 36 × 10⁶ units per month [30]. An East german study stated that rituximab added to first-line mitoxantrone, chlorambucil, and prednisolone chemotherapy followed by interferon maintenance prolongs survival in patients with advanced follicular lymphoma [31]. Another research reported that the role of interferon as initial and maintenance therapy in patients with newly diagnosed FL did not demonstrate any advantage. This study observed stage III or stage IV of 204 patients that receive either chlorambucil (CB), 10 mg daily for 6 weeks, followed by a 2-week interval, with 3 subsequent 2-week treatment periods at the same dose, separated by 2-week intervals, or CB given concurrently with interferon (IFN). IFN was given at a dose of 3 million units thrice weekly, subcutaneously, throughout the 18-week treatment period [32].

4.4. Renal Cell Carcinoma. The definition of renal cell carcinoma (RCC) according to the national cancer institute is a cancer that forms in the lining of very small tubes in the kidney that filter the blood and remove waste products. Long term results of combination of hIFNα2b and interleukin-2 administered subcutaneously in advanced renal cell carcinoma showed an improved survival for advanced RCC [33, 34]. Locatelli’s study [33] stated that objective responses were observed in 9 of 50 (18%) patients and 6 of whom (12%) achieved a complete response. Overall median survival is 12 months; six patients were surviving at a median follow-up of 24 months, and three (6%) are still progression-free. It was also reported that nephrectomy followed by hIFNα2b administration improved survival of 120 metastatic RCC patients [35].

4.5. AIDS-Related Kaposi’s Sarcoma. Kaposi’s sarcoma is a form of skin cancer that can involve internal organs and mostly is found in acquired immunodeficiency syndrome patients. Treatment of Kaposi’s sarcoma with hIFNα2b in 114 patients using three dose regimens, that is, 50 × 10⁶ IU/m² intravenously (high dose), 30 × 10⁶ IU/m² subcutaneously (intermediate dose), or 1 × 10⁶ IU/m² subcutaneously (low dose). Combination low dose of hIFNα2b with zidovudine that administered 10–20 MU day⁻¹ of hIFNα and 500–800 mg day⁻¹ zidovudine in forty AIDS-associated Kaposi’s sarcoma patients. Complete or partial remissions were obtained in 35% of the patients [36].

Eighteen patients (45%) had an overall response (CR + PR) at 3 months and a response persisting for a median of 14 (3–27) months.

4.6. Chronic Myelogenous Leukemia. Chronic myelocytic leukemia (CML) is a cancer of white blood cells in which too many white blood cells are made in the bone marrow. The disease mostly characterized cytogenetically by the presence of the Philadelphia chromosome (Ph) (9;22)(q34;q11) in 90%–95% of patients [38]. A study of safety and efficacy of an indigenous hIFNα2b was conducted in 114 patients with chronic myelogenous leukemia in India. All patients received 5 million units of hIFNα2b daily subcutaneously. The result confirmed that the protein efficacy with Kaplan-Meier probability of survival at 36 months was 76% [39]. Another study reported that low-dose IFN-α2b plus zidovudine seems to be a useful and well-tolerated therapy for KS with antitumoral and antiviral activity. This study observed the effect of combination of low dose of hIFNα2b with zidovudine that administered 10–20 MU day⁻¹ of hIFNα and 500–800 mg day⁻¹ zidovudine in forty AIDS-associated Kaposi’s sarcoma patients. Eighteen patients (45%) had an overall response (CR + PR) at 3 months and a response persisting for a median of 14 (3–27) months [37].
cytogenetic response and patients' survival increased when rhIFN-α-2b was combined with cytarabine [40].

5. Conclusions and Future Directions

rhIFN-α-2b is a protein that has anticancer activity. It has been approved by the FDA as a therapeutic protein that can be used to treat some types of cancer, either in monotherapy or combination therapy with other anticancer drugs. The information regarding the side effects of rhIFN-α-2b needs to be well known because its therapeutic use requires a long time. Development of rhIFN-α-2b to improve the efficacy and safety of proteins is very important. The continuous further exploration on rhIFN-α-2b will lead to the improvement of patient's life quality.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

References


